ALCOHOL WITHDRAWAL-INDUCED HYPERALGESIA

IN YOUNG ADULT BINGE DRINKERS

A Dissertation

by

DOKYOUNG SOPHIA YOU

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Chair of Committee,	Mary W. Meagher
Committee Members,	James W. Grau
	Louis G. Tassinary
	Sherecce A. Fields
Head of Department,	Heather C. Lench

August 2017

Major Subject: Psychology

ABSTRACT

Neuropathy is one of the many health consequences of long-term excessive alcohol consumption. To date, no effective treatment is available for alcoholic neuropathy and its pathogenesis remains unknown. Animal studies have proposed potential mechanisms underlying the association between alcohol abuse and pain conditions. Specifically, preclinical studies have demonstrated that exposure to a few cycles of alcohol withdrawal sensitizes pain pathways and that the two major stress hormones (i.e., epinephrine and cortisol) mediate the induction and maintenance of alcohol withdrawal-induced hyperalgesia. The current study was designed to determine the generality of this phenomenon and mechanisms of alcohol withdrawal-induced hyperalgesia in humans. Because alcohol withdrawal-induced hyperalgesia occurs after only four cycles of an ethanol binge diet in rats, the first objective of the current study was to determine whether alcohol withdrawal-induced hyperalgesia would occur in young adult binge drinkers with a relatively short history of drinking. The second objective was to determine whether stress hormones would be associated with this hyperalgesia. The third goal was to examine the role of negative affect in alcohol withdrawal-induced hyperalgesia because it is a common withdrawal symptom and is linked to enhanced physiological stress responses and pain sensitivity in humans. The last goal was to examine whether alcohol withdrawal and pain would enhance alcohol craving, and therefore, have the ability to motivate continued drinking. To achieve these objectives, two experiments were conducted. Experiment 1 was a cross-sectional design examining the effect of naturally occurring drinking episodes. Experiment 2 was a within-subject design with laboratory alcohol administration. The

current study translated two of the animal findings to humans. First, binge drinkers showed hypersensitivity to muscle pressure pain and alcohol withdrawal further enhanced this hyperalgesia. Second, binge drinkers showed elevated basal levels of epinephrine, but withdrawal did not further increase epinephrine. All the other results were negative. In sum, the current results indicate that alcohol withdrawal-induced hyperalgesia occurs in young adult binge drinkers with a relatively short history of binge drinking and even before the development of alcoholic neuropathy. The study also suggests epinephrine may play a role in alcohol withdrawal-induced hyperalgesia.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Meagher, and my committee members, Dr. Grau, Dr. Tassinary, and Dr. Fields, for their guidance and support throughout the course of this research. Additionally, I thank Dr. Thomas H. Welsh for his training in analyzing and interpreting an enzyme-linked immunosorbent assay.

Thanks also go to my undergraduate research assistants, laboratory technicians, and laboratory colleagues for their contribution to this research project. I also want to extend my gratitude to the National Institute on Alcohol Abuse and Alcoholism, and the Texas A&M University College of Liberal Arts, for providing financial support for my graduate study and this research project.

Finally, thanks to my family for their encouragement, patience, and love.

CONTRIBUTORS AND FUNDING SOURCES

Contributors

This work was supervised by a dissertation committee consisting of committee chair, Professor Mary Meagher, of the Department of Psychology and Institute for Neuroscience (the student's advisor), Professors James W. Grau and Sherecce Fields (also of the Department of Psychology and Institute for Neuroscience), and Professor Louis Tassinary, of the Department of Visualization, College of Architecture.

Funding Sources

Graduate study was supported by an NRSA Predoctoral Fellowship from the National Institute on Alcohol Abuse and Alcoholism (NIAAA). This work was made possible in part by the Dissertation Enhancement Award from the College of Liberal Arts, Texas A&M University.

TABLE OF CONTENTS

LIST OF FIGURES

Figure Page

LIST OF TABLES

1. INTRODUCTION

Alcohol is the most widely used substance. According to a 2014 national survey, about half of Americans aged 12+ drink alcohol (1). This survey also reveals that binge (23%) and heavy drinking (6%) are common. Across different age groups, binge drinking is much more common for young adults (38%) compared to adolescents (6%) and adults (23%) (1). The National Institute on Alcohol Abuse and Alcoholism (NIAAA) defines binge drinking as 4(women)/5(men) drinks in about 2 hours. This pattern of drinking, a large amount of alcohol in a short time, brings blood alcohol concentration (BAC) levels to at least 0.08%. Heavy drinking is defined as 5+ drinks on an occasion at least one day in the past 30 days. Excessive drinking in the form of binge or heavy drinking leads to many physical and mental health problems (2). Painful neuropathy is one of the health problems after long-term excessive use, but can develop for some after a relatively short period of alcohol abuse: 20% in the first 5 years and 40% after 10 years of alcohol abuse (3). The pathogenesis of alcohol-induced painful neuropathy is largely unknown and no effective treatment is currently available. Therefore, systematic investigation of its mechanisms is a critical first step toward designing more effective treatment approaches.

Studies examining the relationship between alcohol and pain have found that alcohol has both analgesic and hyperalgesic properties. Alcohol's analgesic property has been well established in humans. Wolff and colleagues have systematically examined the analgesic effect of alcohol in the 1940s, demonstrating that drinking 15 to 90cc of 95% of alcohol quickly raises heat pain threshold within 10 minutes (4). The peak analgesic effects occur within 40 minutes for small amounts of alcohol (70cc) and 15 minutes for

the larger amounts (90 cc). The maximum analgesic effect is a 45% increase in heat pain threshold with 30cc of 95% alcohol and there are no further analgesic effects with additional amount of alcohol (i.e., 40-90cc). The analgesic effect from a large amount of alcohol disappears within 4 hours and therefore, it is considered a short-term analgesic (4). Some argue that alcohol produces inadequate antinociceptive effects to be a useful analgesia, but it is effective in reversing withdrawal-induced hyperalgesia (5). This reversal suggests alcohol may be a negative reinforcer for continuous drinking.

Although alcohol's hyperalgesic properties have not received much attention in human studies, alcohol withdrawal-induced hyperalgesia is a well-established phenomenon in animal models of binge drinking. After one cycle of an ethanol binge diet (4 days on/3days off), rats develop hyperalgesia to pressure pain on the dorsum of the hind paw during ethanol withdrawal (6). This hyperalgesia worsens with repeated cycles of an ethanol binge diet up to 4 weeks and persists up to 5 weeks at the time of study termination. More importantly, this study has shown the critical role of withdrawal in the development of hyperalgesia (6). Contrary to ethanol-binge diets, a continuous ethanol diet (7days on/0 days off) reduces pain threshold much later (i.e., after 4 cycles) to a small degree. Additionally, switching to a continuous ethanol diet (7days on/0 days off) after one cycle of an ethanol binge diet (4 days on/3days off) does not further decrease pain threshold. This suggests that repeated alcohol withdrawals, not excessive use of alcohol, contribute to the development of and further worsening of hyperalgesia. Furthermore, fewer days on an ethanol diet (1, 2, or 3 days on/3 days off) produce rapid and greater hyperalgesic effects than more days on an ethanol diet (4 days on/3days off). This indicates that

consuming alcohol for more consecutive days does not contribute to hyperalgesia, but rather produces a protective effect. This protective effect of alcohol possibly motivates continued drinking despite multiple physical and psychosocial problems that result from excessive drinking.

A human study has also shown evidence for alcohol withdrawal-induced hyperalgesia (7). Middle-aged men with alcohol dependence, but without other physical problems (e.g., diabetes, liver cirrhosis, and peripheral neuropathy) show reduced heat pain thresholds and tolerances on the left volar wrist and sternum during hospitalization for acute alcohol detoxification (7). This hyperalgesia is reversible with alcohol abstinence. This human study indicates that alcohol withdrawal-induced hyperalgesia can occur in those with alcohol dependence even before the occurrence of alcoholic neuropathy. However, it remains unclear whether alcohol withdrawal can induce hyperalgesia in those who have a relatively short history of binge drinking and even before the development of alcohol dependence.

Changes of pain sensitivity occur during two phases: acute and chronic. The acute phase refers to changes in pain sensitivity during acute alcohol withdrawal, and the chronic phase refers to persistent changes in the absence of alcohol consumption. For the acute phase, the time of the withdrawal-induced hyperalgesia seems to vary depending on testing method (pain modality) and drinking patterns. According to an animal study, heat hyperalgesia on the tail flick tests emerges at 3 hours, peaks between 6 and 12 hours, and disappears within 36 hours after stopping a 10-day ethanol diet (8). Another animal study shows inflammatory hyperalgesia on the formalin test at 12 hours after stopping a 10-day

ethanol diet (9) and heat hyperalgesia on the tail flick and hot plate tests at 24 hours after stopping a 7-day of ethanol diet. Unlike the former study with tail-flick test (8), the latter studies have not explored the time course of the development of withdrawal-induced hyperalgesia. Compared to withdrawal after a consecutive ethanol diet for 7 or 10 days, withdrawal after an ethanol binge diet (4 days on/3 days off) produces more persistent hyperalgesia in paw-pressure tests (6). Specifically, hyperalgesia is observed at 12 hours, peaks at 60 hours, and lasts for 7 days after stopping the ethanol diet (6). The time course of hyperalgesia during alcohol withdrawal in humans is unknown, but the symptoms of alcohol withdrawal have been well characterized. Therefore, the withdrawal symptoms will be reviewed next to estimate the potential time window for alcohol withdrawal-induced hyperalgesia.

DSM-5 diagnostic criteria for alcohol withdrawal syndromes are two or more of the following symptoms within several hours to a few days after cessation or reduction of alcohol use. Such symptoms are a) insomnia, b) autonomic hyperactivity (e.g., sweating or heart rate > 100 bests/min), c) increased hand tremor, d) nausea or vomiting, e) psychomotor agitation (physical restlessness), f) anxiety, g) hallucinations or perceptual disturbances of the auditory, tactile, or visual type, and h) grand mal seizure (10). Minor withdrawal symptoms generally occur within 6 to 12 hours after the drinking episode, followed by hallucination (12-24 hours), withdrawal seizures (24-48 hours), and delirium tremens (48-72 hours), summary in Fig. 1 (11).

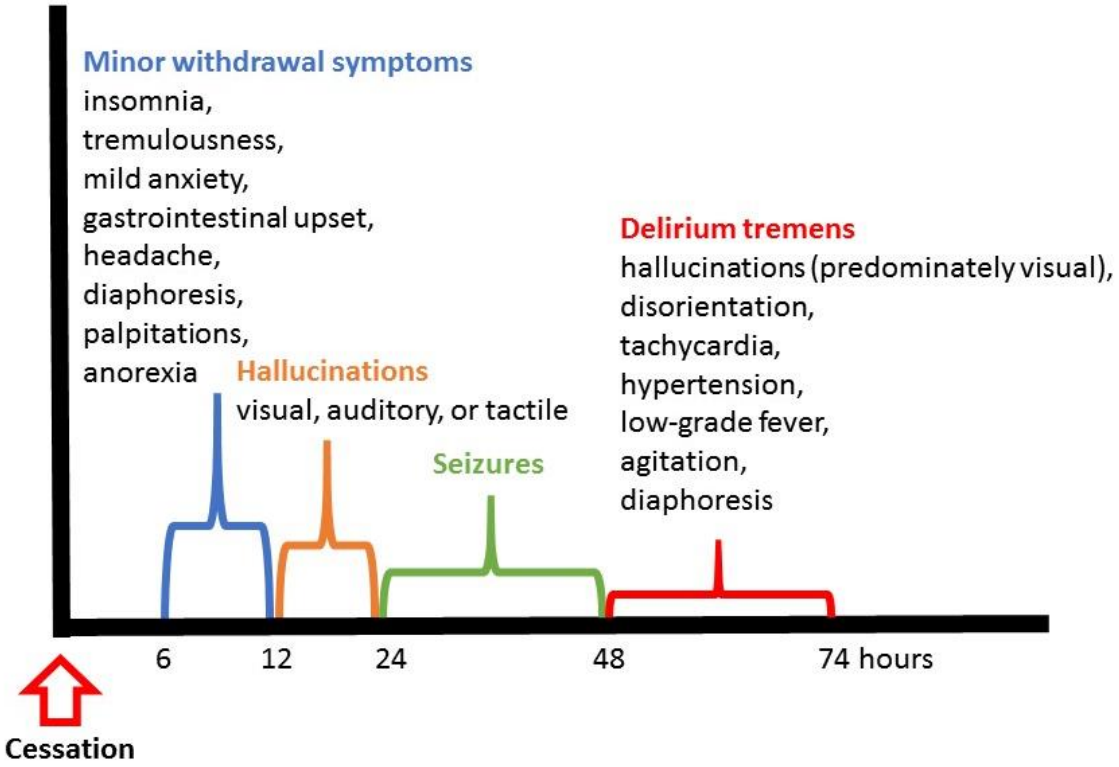

Fig. 1. Timeline of alcohol withdrawal symptoms

In the early stages of binge drinking, withdrawal symptoms are commonly called hangover symptoms. A study examining hangover symptoms after naturalistic drinking at a Danish beach has shown that hangover symptoms occurred in 68% of 112 young adults after drinking at least 12 standard drinks and that symptom severity is dose-dependent and increases over time with repetitive alcohol consumptions (12). An alcohol administration study in Sweden shows a similar finding. Of 172 adults who drank alcohol to 0.10% BAC (7:30pm - 10pm), 76% reported hangovers on the following morning at 8am (13). The most common symptoms were thirst (100%), tiredness (98%), headaches (66%), dizziness/faintness (50%), loss of appetite (45%), stomachache (26%), nausea (25%), and

heart racing (18%), but emotional states and typical drinking patterns were not evaluated in this study (13). Others have observed elevated anxiety symptoms in the morning after drinking 3.25ml/kg body weight of bourbon or vodka from 8pm to midnight (14). In this study, the BAC was on average 0.093% immediately after drinking and at minimal levels (0.005% for bourbon and 0.007% for vodka) on the following morning, 8 hours after drinking. Alcohol withdrawal and hangover symptoms are similar in that headaches, fatigue, and poor sense of overall well-being are common in both conditions, but are dissimilar in that hallucinations and seizures are uncommon in hangover (15,16). Additionally, the duration of hangovers is shorter whereas withdrawal symptoms last up to 5 days after drinking (16). Across these different studies, hangover symptoms are evaluated within 24 hours after drinking and hyperalgesia may occur within this timeframe.

In addition to the time course, the mechanisms of alcohol withdrawal-induced hyperalgesia are understudied in humans. In an animal model of ethanol binge diets, Dina and colleagues have found two stress hormones as the mediators of withdrawal-induced hyperalgesia (17). Their data show that an ethanol binge diet increases plasma epinephrine levels about 1.8 times by the third week and the level is further elevated about 1.9 times during alcohol withdrawal, 18 hours after the ethanol diet (17). This elevated epinephrine plays a critical role in the induction and maintenance of the hyperalgesia. Blocking epinephrine by adrenalectomy prevents the occurrence of alcohol withdrawal-induced hyperalgesia and reverses the established hyperalgesia despite the continuation of the ethanol binge diet (17). Then, a constant infusion of epinephrine reinstates the

hyperalgesia within a week (17). Plasma cortisol also plays a similar role. Administration of a glucocorticoid receptor antagonist prevents and reverses the withdrawal-induced hyperalgesia (17). These results suggest the involvement of the sympatho-adrenal medullary (SAM) and hypothalamo-pituitary-adrenal (HPA) stress axes in the induction and maintenance of the hyperalgesia.

A review of animal findings explains other potential mechanisms involved in the induction of withdrawal-induced hyperalgesia: $GABA_A$, calcium channels, adenosine receptors, analgesics, NMDA and other glutamate receptors, and signal transduction pathways (5). $GABA_A$ antagonists, calcium channel blockers, and adenosine antagonists prevent or attenuate the development of ethanol-withdrawal hyperalgesia, but they do not reverse the hyperalgesia. COX-2 selective NSAIDs also prevent ethanol withdrawal-hyperalgesia, but reversal has not been investigated. Although not being tested in intact animals, ethanol withdrawal-induced hyper-excitability of NMDA receptors has been observed in the spinal cord (5). Lastly, protein kinase C epsilon (PKCε) is involved in the induction and maintenance of withdrawal-induced hyperalgesia (6,18). PKCε, a cellular signaling pathway, is known to play an important role in mechanical hyperalgesia in models of inflammatory, neuropathic and generalized widespread pain (19). Levels of expression of PKCε in the dorsal root ganglia are increased by 50% after a chronic ethanol diet (18). Intrathecal injection of an antisense oligonucleotide against PKCε reduces hyperalgesia in the induction and maintenance phase (6). Consequently, the PKCε signaling pathway and the above-mentioned mechanisms are possibly involved in alcohol withdrawal-induced hyperalgesia in humans. To date, studies have focused on the

peripheral and spinal cord mechanisms and little is known about the supra-spinal mechanisms.

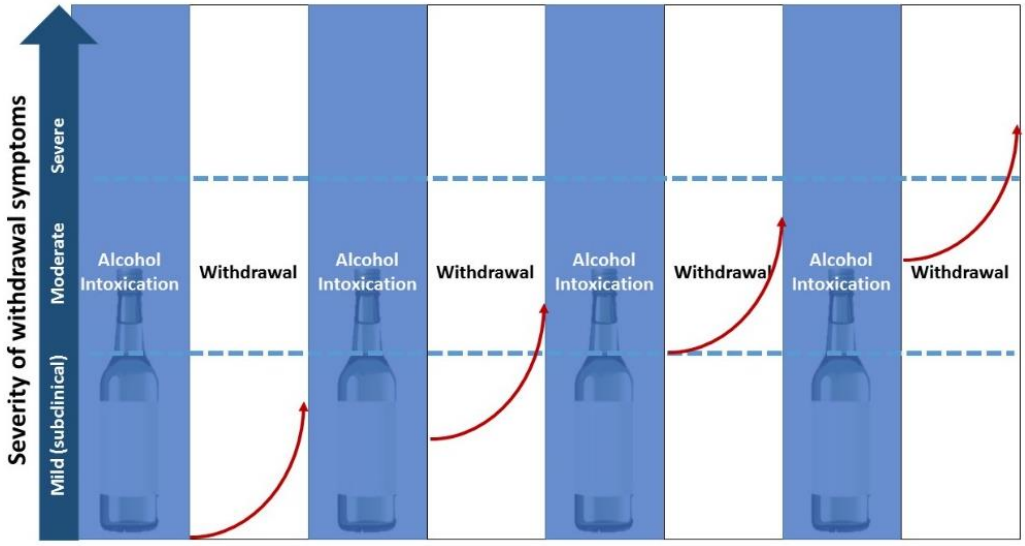

Fig. 2. Becker (1998)'s graphical presentation of kindling process, modified (Reprinted, public domain document)

Alcohol suppresses the central nervous system (CNS) functions. When alcohol levels decline during withdrawal, the CNS hyperexcitability occurs as a compensatory response (20). This CNS hyperexcitability manifests as withdrawal symptoms of tremors, agitation, seizures, and delirium tremens (20). Although these withdrawal symptoms are not common in early stages of binge drinking, repeated alcohol withdrawal episodes sensitize people to subsequent withdrawal episodes and withdrawal symptoms worsen over time. Kindling may explain increased neuronal excitability with repeated alcohol withdrawal episodes, Fig. 2 (20). Withdrawal-induced brain function changes are characterized by imbalance of inhibitory/excitatory neurotransmission (↓ $GABA_A$

receptor, altered adenosine, ↑ NMDA receptor, ↑ voltage-gated C channel functions), neuroendocrine dysregulation (↑ HPA axis activation), and other neurochemical perturbations (↓ dopamine function) (20). As a result of kindling, seizure susceptibility, anxiety, and neurotoxicity are increased and the subjective perception of alcohol effect changes over time.

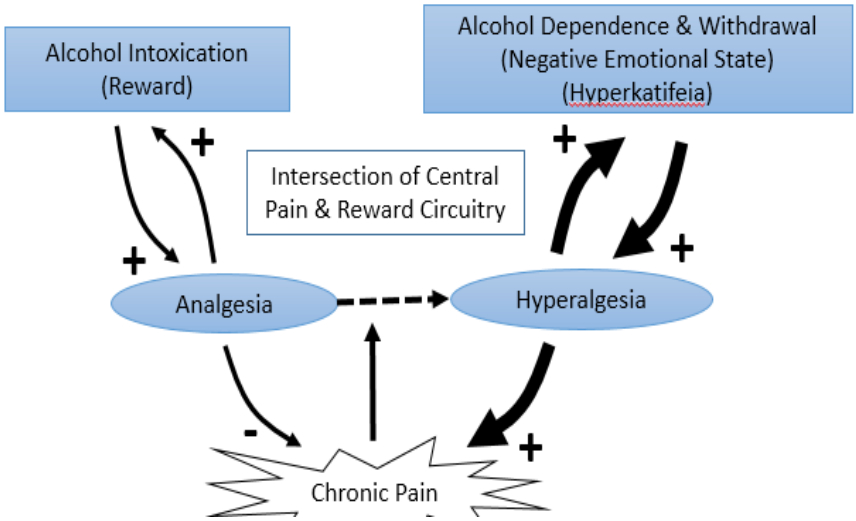

Fig. 3. Egli *et al*. (2012)'s schematic diagram describing the bi-directional relationship between alcohol use and chronic pain. (Reprinted, public domain document)

Alcohol's immediate analgesic effect and the delayed onset of the hyperalgesic effect during withdrawal create a vulnerability to alcohol dependence, Fig. 3 (21). The immediate reward of an analgesic effect can motivate drinking. Here, alcohol is a positive reinforcer. Following opposing dysphoric and hyperalgesic acute withdrawal states can also motivate drinking to reduce emotional distress and pain. In this case, alcohol is a negative reinforcer. Because withdrawal symptoms are intensified with repeated binge drinking, alcohol's negative reinforcing effect becomes more critical in continued

drinking. Especially, persistent negative affect and hypersensitivity to emotional distress (hyperkatifeia) during withdrawal become strong motives for drinking in alcoholics (21).

Egli and colleagues suggest that alcohol dependence is a chronic pain disorder because affective (hyperkatifeia) and sensory components of pain (hyperalgesia) could promote excessive drinking (21). Additionally, the dysregulated reward and emotional neural circuits found in chronic pain conditions and alcohol dependence could alter central pain processing and alcohol drinking patterns (22). Specifically, the dysregulated reward system involves increased connectivity between nucleus accumbens and medial prefrontal cortex (mPFC), indicating over-activation of the brain reward system. Dysregulation of the emotional neural circuit leads to increased activity in the amygdala and impaired function of the mPFC, which are associated with more negative affect and less top-down cognitive control of pain and alcohol use.

Persistent pain and repeated episodes of excessive alcohol intoxication and withdrawal can also alter the brain stress circuit (signaling corticotropin-releasing factor [CRF] in the central nucleus of the amygdala) (22). Koob and Le Moal introduce a concept of anti-reward system to explain the transition from substance use to abuse or dependence. To oppose the over-activation of the brain reward system from binge drinking, the anti-reward system (i.e., the brain stress system) is recruited as part of a neuroadaptation process (23). Consequently, the combination of a reduced reward system (decreased dopamine and euphoria) and an enhanced anti-reward system (increased CRF and increased dysphoria and distress) produces a powerful motivational state for addiction. Eventually, this dysregulation of the motivation systems results in a switch from impulsive

substance use to compulsive use (Fig. 4). Under the influence the anti-reward system, stress and negative affect not only can promote drinking, but also can produce pain hypersensitivity (22).

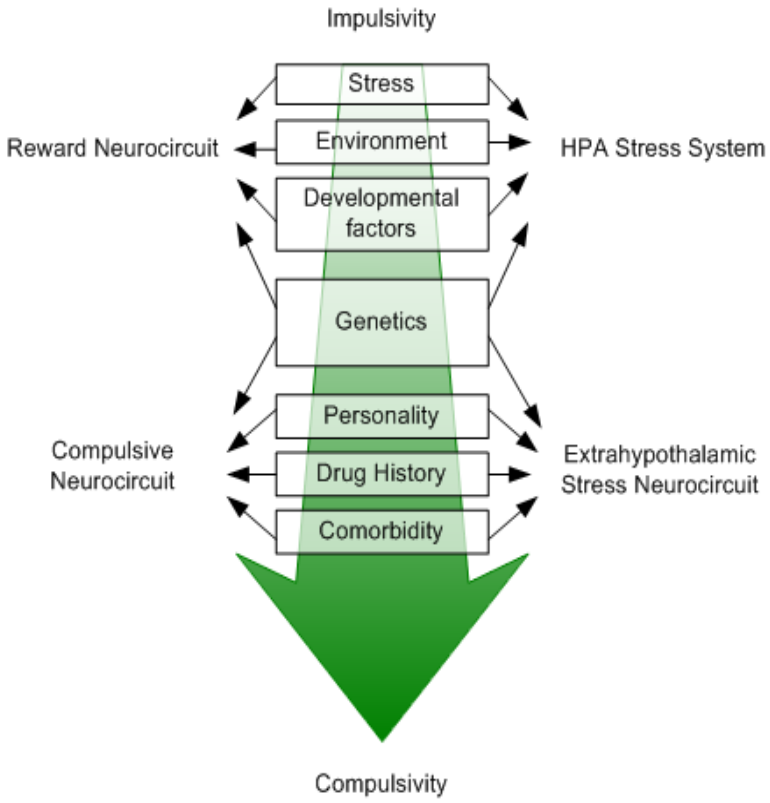

Fig. 4. Koob and Le Moal (2008)'s hypothetical diagram illustrating sources of vulnerability in addiction (Reprinted with permission by the authors)

This review of the literature suggests that excessive drinking and pain influence each other and identifies several mechanisms that are proposed to be tested in humans. As an initial effort to unravel this complex relationship, the current study focused on the effect of alcohol-withdrawal on pain sensitivity, the role of stress hormones and negative affect

in withdrawal-induced hyperalgesia, and pain as a potential motivational force for drinking. Specific objectives were as follows. The first objective was to examine whether alcohol withdrawal induced hyperalgesia would occur in young adult binge drinkers who would have a relatively short history of binge drinking and therefore would have relatively less episodes of alcohol withdrawal. The second objective was to examine whether stress hormones (i.e., epinephrine and cortisol) would be correlated with drinking patterns. Third, this study examined whether negative affect (i.e., anxiety) would enhance alcohol withdrawal-induced hyperalgesia. Finally, this study examined whether pain and alcohol-withdrawal would enhance alcohol craving and therefore potentially motivate drinking. To achieve these objectives, two experiments were conducted. Experiment 1 was a cross-sectional study examining peripheral pain sensitivity after naturally occurring alcohol use. Experiment 2 was a within-subject design examining capsaicin-induced pain sensitivity with laboratory alcohol administration. Capsaicin was used in Experiment 2 to examine various characteristics of inflammatory-mediated pain processing (24). Capsaicin is an active ingredient of hot chili pepper and selectively activates transient receptor potential vanilloid 1, which locally releases various pro-inflammatory neuropeptides such as substance P and calcitonin gene-related peptide (24). Consequently, topical capsaicin temporarily induces spontaneous burning pain on the capsaicin application site, flare, and primary and secondary hyperalgesia. Primary hyperalgesia is enhanced pain sensitivity on the affected site and secondary hyperalgesia is enhanced pain sensitivity on the adjacent unaffected sites. Different from the other pain measures, the results of secondary

hyperalgesia as a proxy measure for central sensitization would inform whether alcohol withdrawal would alter central pain processing (25,26).

It was hypothesized that binge drinkers would show pain hypersensitivity and this hypersensitivity would be further enhanced during acute alcohol withdrawal. Additionally, it was hypothesized that stress hormones would be increased in binge drinkers and be further amplified during acute alcohol withdrawal; that more negative affect during alcohol withdrawal would be associated with enhanced hyperalgesia; and that alcohol withdrawal and pain during alcohol withdrawal would increase craving.

2. EXPERIMENT 1

Texas A&M University Institutional Review Board approved the study procedures and informed consent was obtained from all participants. Participants were recruited from the Summer 2014 to the Fall 2015. Participants were eligible for inclusion if they were healthy young adults between 18 and 30 years old. Exclusion criteria were any chronic health issues including chronic pain, prescription medication use (except contraceptives), history of vasovagal syncope, and needle phobia.

Potential participants completed an online prescreening survey (Qualtrics.com) evaluating health status and drinking history. Typical drinking patterns were assessed by a statement, "Select one that describes your alcohol drinking," with options never, former, and current drinker. Those who endorsed "Never" (57%) were classified as abstainers. Those who endorsed as "former" drinkers (6%) were not invited. Those who endorsed being "current" drinkers (37%) were asked to complete the Daily Drinking Questionnaires (DDQ) (27). On the DDQ, participants were asked to report the number of standard drinks and hours spent drinking for a typical and heaviest drinking week in the last 30 days. Those who endorsed as current drinkers, but reported no alcohol use in the past 30 days were not invited. Individuals who reported their maximum alcohol consumption less than 4(women)/ 5(men) standard drinks per episode were invited as moderate drinkers. Individuals who reported their typical alcohol consumption as at least 4(women)/ 5(men) standard drinks for 2 hours were invited as binge drinkers. Heavy, but not binge, drinkers defined by at least 4 (women)/5 (men) standard drinks over more than 2 hours were not invited.

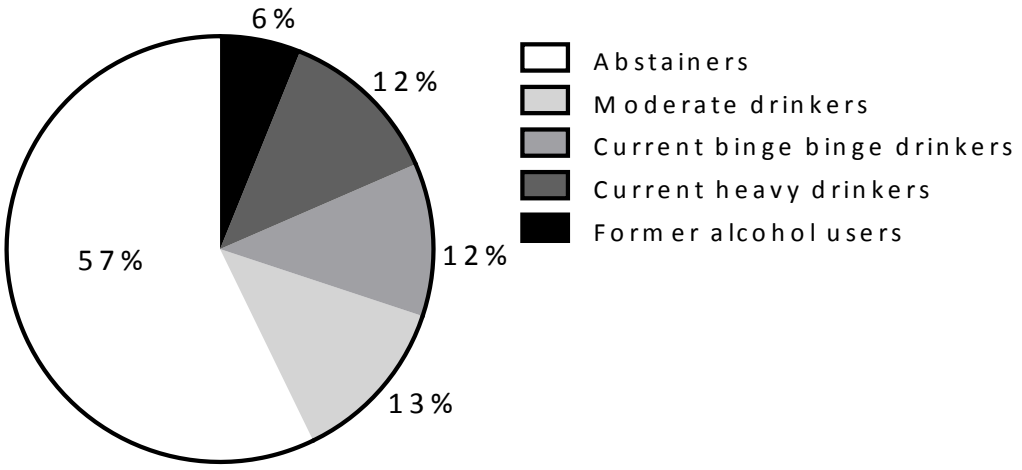

Fig. 5. Prevalence of different drinking patterns in young adults

Of the total 1,562 women and 664 men who completed the prescreening (M_{age} = 19.4, SD_{age} = 2.1, 18-30 years), 13% were abstainers, 12% were binge drinkers, and another 12% were heavy drinkers regardless of eligibility criteria (Fig. 5). About a third (29%) were not invited due to meeting ineligibility criteria.

Of the total 1,573 eligible participants who were invited via email, 177 people participated in this study and all consented participants completed the laboratory experiment. Prior to the laboratory visit, scheduled individuals were instructed not to take pain or allergy medicine 3 days before the experiment and not to drink caffeine or to exercise vigorously 4 hours before the experiment. Participants were instructed to reschedule the visit if they had an injury or skin condition on the feet (QST testing site) or acute illness on the day of the experiment. Compliance to these study preparations was verbally verified at the beginning of the experiment. In case of non-compliance, the experiment was rescheduled. Participants' drinking patterns were assessed again in the

laboratory after the sensory testing and actual drinking patterns were modified as necessary. Most participants (85%) received class credit for their participation and the rest participated to be entered in a raffle to win one of the three $100 gift cards.

3. METHODS

EXPERIMENT 1

3.1 Measures

The following measures were administered to characterize binge drinkers' psychological traits as well as to examine the roles of negative affect in alcohol-withdrawal induced hyperalgesia.

Self-reports

The **Perceived Stress Scale** (PSS) is a 10-item measure used to assess levels of perceived life stress in the last month (Cronbach's α =.78) (28). The scale ranges from 0: *Never* to 4: *Very Often.* Of the 10 items, four items are reversed coded. The total scores range from 0 to 40 with higher scores indicating higher levels of perceived stress. This scale has been validated in college students as well as in the general population (28). The reported norms for young adults is 14.2 with *SD* of 6.2. The average PSS score of the current sample was 16.6 (*SD* = 6.0), which was higher than previously observed norm ($t = 4.59$, $p < .001$).

The **Depression Anxiety Stress Scales** (DASS) is a 21-item questionnaire to assess the symptom severity of depression (Cronbach's α =.94), anxiety (Cronbach's α =.87), and life stress (Cronbach's α =.91) over the past week for a community sample (29). This measure uses a 4-point Likert scale, ranging from 0: *Did not apply to me at all* (Never) to 3: *Applied to me very much, or most of the time* (Almost Always). Seven items were used to evaluate each symptom domain. The severity of each symptom is calculated

by multiplying the summed scores by 2. Therefore, the total scores range from 0-42. Reported normal ranges are less than 10 for depressive symptoms, less than 8 for anxiety symptoms, and less than 15 for stress (30). The respective averages scores of DASS-depression, anxiety, and stress of the current sample were 5.6 (*SD* = 6.3), 4.7 (*SD* = 4.7), and 9.2 (*SD* = 6.4). Therefore, our participants' symptoms were all on average within the normal limits.

The **Alcohol Craving Questionnaire** (ACQ-NOW)-short form is a self-report questionnaire used to assess levels of alcohol craving. Of the 12 items, 8 items were reversed coded before summing up to compute the levels of craving. The items are scored on a 1: *strongly disagree* to 7: *strongly agree* scale. This measure evaluates four dimensions, with each dimension having 3 items: emotionality (negative emotional state, Cronbach's α =.86), purposefulness (desire and intent to drink right now, Cronbach's α =.77), compulsivity (lack of confidence in ability to quit drinking, Cronbach's α =.79), and expectancy (positive alcohol expectancy, Cronbach's α =.77) (31).

The **Barratt Impulsiveness Scale** (BIS-11) is a self-report measure to assess levels of impulsivity (Cronbach's α =.82 for undergraduates) (32). The scale is composed of 30 items (10 revered coded items) and each item is scored on a 4-point scale from 1: *Rarely/Never* to 4: *Almost always/Always*. The total scores range from 30 to 120 with higher scores indicating greater impulsivity. Additionally, the BIS-11 has three subscale scores: inattention (8 items), motor restlessness (11 items), and non-planning (11 items).

The **Hangover Symptoms Scale** (HSS) is a 5-point, self-report to assess the frequency of 13 hangover symptoms over the past 12 months (33). The scale ranges from

0 (0% of the time) to 4 (100% of the time). The items were dichotomized to identify presence (1 = 1 – 4) or absence (0 = 0) of the symptom and then, summed. Therefore, the possible total scores range from 0 to 13, with higher scores indicating more hangover symptoms (Cronbach's α =.83 for men and .84 for women) (33). This measure was administered in the prescreening questionnaire to evaluate typical hangover symptoms and to compare acute hangover symptoms after the laboratory pain testing.

The **Acute Hangover Scale** (AHS) is a self-report measure used to assess acute hangover symptoms (Cronbach's α = .84) (34). The 9 items of hangover symptoms, thirsty, tired, headache, dizziness, loss of appetite, stomachache, nausea, and heart racing are rated on a 0: *None* to 7: *Incapacitating* scale. The average AHS total scores are 1.4 (*SD* = 0.9) on the morning after alcohol intoxication and 0.6 (*SD* = 0.4) on the morning after placebo (34).

The following measures were administered to evaluate emotional responses to pain testing.

The **Spielberger State-Trait Anxiety Inventory-6** (STAI) is a 6-item measure used to evaluate levels of state anxiety (Cronbach's α =.82) (35). The items are scored on a 4-point scale; 1 *Not at all* to 4: *Very much*. The total scores range from 6 to 24 with higher scores indicating higher levels of anxiety.

The **Self-Assessment Manikin** (SAM) is a 9-point scale pictogram measuring affective dimensions of valence, arousal, and dominance (36). The valence scale ranges from 1: *Happy* to 9: *Unhappy*, the arousal scale ranges from 1: *Calm* to 9: *Excited*, and the dominance scale ranges from 1: *Feeling being controlled* to 9: *Feeling in control*.

The **Positive and Negative Affect Schedule** (PANAS) is used to evaluate two dimensions of current mood such as positive and negative affective states (37). The PANAS consists of two 10-items for positive (PA) and negative affect (NA). The possible total scores are between 10 and 50 for positive (Cronbach's $\alpha = .85$) and negative affect (Cronbach's $\alpha = .89$) (37). Higher scores indicate more positive or negative affect.

Quantitative Sensory Testing (QST)

All pain testing was conducted in a sound-attenuated and temperature controlled room (20-25 ºC). Because alcohol-induced polyneuropathy often affects the nerves in legs or feet (38,39), pain testing was conducted on the dorsum of the non-dominant foot (L5 dermatome, Fig. 6). Non-dominant foot was identified by asking a question, "which foot do you use to kick a ball?" If response was unknown or equal, the foot with a smaller circumference of the calf muscle was considered to be non-dominant. A modified Quantitative Sensory Testing (QST) protocol of the German Research Network on Neuropathic Pain was used to measure mechanical, heat, and pressure pain thresholds (40). The details of the QST protocol are described below.

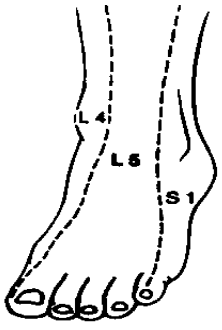

Fig. 6. L5 dermatome

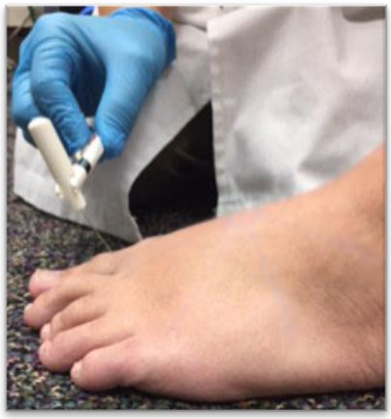

Fig. 7. Von Frey application

The **mechanical pain threshold** was measured using the method of limits (40). Fixed stimuli with a set of seven Von Frey filaments (0.6, 1.4, 4, 6, 15, 26, and 60gF, Stoelting Co. Wood Dale, IL, USA) were applied at a rate of 2s on and 2s off in an ascending order until participants first perceived a von Frey stimulus as being painful. Then, filaments were applied in a descending order until the first perception of no pain was reported. Von Frey is a calibrated monofilament that bends at a certain pressure. An experimenter puts a von Frey filament on a skin at a 90º angle and slowly increases the pressure until it bends (Fig. 7). To void sensory fatigue and local sensitization, a von Frey filament was applied to a different location and the time interval between one trial and another was at least 10s. The mechanical pain threshold on feet (using a 0.2 diameter von Frey) is reported to be on average 9.0gF (*SD* = 7.6) (41).

The Geometric mean of the three series of ascending and descending stimulus intensity was used to calculate individual mechanical pain threshold. When some participants reported no painful sensation with the highest force (60gF), the next available

highest force (100gF) was used as their pain threshold. The German protocol uses a customized von-Frey with a diameter of 0.2mm whereas the diameters of the commercially available von Frey (Stoelting Co.) vary across different forces. Specifically, respective diameters of the seven von Frey filaments were 0.20, 0.25, 0.36, 0.38, 0.48, 0.56, and 0.71 mm in an ascending order.

The verbal script for the instruction was as follows: *Different filaments will be applied to your foot. If you feel a sharp pain sensation, please indicate "NOW." After a couple seconds, filaments will again be applied to your foot. At that time, if you first feel no pain, please indicate "NOW." We are going to repeat this sensory test three times.*

Fig. 8. Thermode application

The **heat pain threshold** was measured using a 9 cm^2 Peltier thermode (ATS, Medoc Ltd., Israel, Fig. 8). Temperature was ramped at 1ºC/s (baseline temperature 32ºC) until participants indicated their first pain sensation or temperature reached to 52ºC. This method succeeded in detecting the threshold in all participants. The interval between the tests was 30s. The reported means of the heat pain threshold on the dorsum foot were 43.8ºC (*SD* = 2.8) for young women and 45.1ºC (*SD* = 2.4) for young men (41). Another

study reports the median of the heat pain thresholds on the dorsum foot is 43.7 with the 5th-95th percentiles of 38.3-47.6ºC (42).

The verbal script for the instruction was as follows: *You will feel a heat stimulus on your foot. The thermode will start at a low temperature and the temperature will slowly increase over time. Please respond by clicking the mouse as soon as the stimulus becomes painful. Remember, your first instinct may be to respond when the heat first turns on, but try to concentrate on clicking it only when you first feel pain. Remember to look at the black dot in front of you throughout the test. The test will only take a few minutes.*

The **pressure pain threshold** test was conducted using a handheld algometer (FPX 50 model, Wagner Instrument, CT, USA). An experimenter put a 1cm^2 diameter rubber tip on a skin at a 90º angle (Fig. 9). Then, force was increased by 50kPa/s (~0.5kg/cm^{2}s) until participants indicated that they first felt pain.

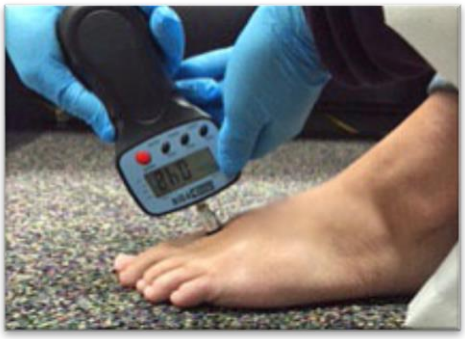

Fig. 9. Algometer application

The verbal script for the instruction was as follows: *You will feel pressure on your foot. The pressure will slowly increase over time. Please say "STOP" as soon as the*

stimulus becomes painful. Remember to look at the black dot in front of you throughout the test. The test will only take a few minutes.

Enzyme-linked immunosorbent assay (ELISA)

All samples were only thawed once before the analysis and duplicate samples were analyzed according to the manufactures' manual. Abnova (Taipei, Taiwan, Cat. No KA1877) and IBL international (Hamburg, Germany, RE52061) kits were used for Epinephrine and Cortisol, respectively. Pre- and post- samples from the same subjects were all analyzed in the same plate. Although an attempt was made to reduce plate to plate variability by analyzing the same number of participants from each group in one plate, it was not entirely possible due to unequal *n*. Below, the ELISA procedures are briefly described.

Before the **Epinephrine ELISA** procedures, sample preparation, extraction, and acylation were conducted at room temperature. Two 300µL aliquots of plasma were used for duplicate tests. Then, 250µL of distilled water, 50µL of assay buffer, and 50µL of extraction buffer were added into the respective wells of the extraction plate and the plate was incubated for 30 minutes on a shaker (VWR Model 3500). After the incubation, the plate was decanted and 1ml of wash buffer solution was added into each well. After this wash step was repeated one more time, 150µL of acylation buffer and 25µL of acylation reagent were added into each well and the plate was incubated for 15 minutes on a shaker. After emptying the plate, 1ml of wash buffer was added into all wells and the plate was incubated for 10 minutes on a shaker. After the incubation, the plate was decanted and was incubated again for 10 minutes with 150µL of hydrochloric acid.

The ELISA procedure was also performed at a room temperature. First, 25μL of the enzyme solution was added into all wells of the epinephrine microtiter plate. After adding 100μL of the extracted standards, controls, and samples into each well, the plate was incubated for 30 minutes on a shaker. The plate was incubated again for 2 hours with 50μL of epinephrine antiserum, followed by three wash steps. Then, 100μL of the enzyme conjugate was added into all wells and the plate was incubated for 30 minutes on a shaker. After the incubation, the plate was washed three times and was incubated for 30 minutes with 100μL of the chromogenic substrate on a shaker. Then, 100μL of the stop solution was added and the optical density measures were read at 450nm on a Victor X3 Multilabel Plate Reader (PerkinElmer, Waltham, Massachusetts, USA). Finally, individual epinephrine levels were calculated by four-parameter logistic regression (www.myassay.com). The total assay time per plate from the sample preparation to ELISA was about 9 - 10 hours. According to the manual, the reference range of the plasma epinephrine is between 18 and 6,667 pg/mL. Intra- and inter-assay Coefficient of Variability (CV)s range from 11.0 to 24.7%.

The **cortisol ELISA** was conducted using two 20μL aliquots of plasma. After putting 200μL of enzyme conjugate into each well, the plate was placed at room temperature for an hour incubation, followed by three wash steps. Then, 100μL of TMP substrate was added into each well and the plate was incubated for 15 minutes at room temperature. After adding 100μL of TMB stop solution into each well, the optical density measures were read at 450nm. Individual cortisol levels were calculated by a four-parameter logistic regression (www.myassay.com). The total assay time per plate was

about 2.5 hours. The reference range of the plasma cortisol in the afternoon is 30-160ng/mL (43). Intra- and inter-assay CVs range from 2.1 to 5.0%.

Physiological Measures

Continuous heart rate (HR), skin conductance level (SCL), and respiration rate (RR) were measured using a MP150, BIOPAC Systems, Inc. (Goleta, CA, USA), interfacing with AcqKnowledge software 4.2 for data acquisition. The sampling rate was 1000Hz. LabVIEW 8.0 was also used to generate a digital signal from the National Instrument 6008USB device (Austin, TX USA) to STP100C, connecting to UIM100C (both BIOPAC Systems, Inc). Blood pressure was measured before and after the QST.

A finite impulse response (FIR) band pass filter was used to remove the motion noise in HR (0.5 and 35Hz) (44) and RR (0.05 and 1.0 Hz) before calculating the rates. To remove artifact on SCL, 1 Hz FIR low pass filter was used. After filtering, all data were visually inspected. Filtering corrected most noise in RR and SCL. However, three subjects' HR data during or after heat pain threshold testing were not analyzed for uncorrectable noises (e.g., extreme motion artifacts, detached sensors).

3.2 Procedures

Four female experimenters conducted this experiment. Two experimenters were involved in prescreening, scheduling, and providing preparation instruction. To increase the probability of recruiting individuals who consumed alcohol within 48 hours (i.e., the acute alcohol withdrawal phase), there was no preparation instruction about alcohol consumption, and the experiment was conducted from Thursday through Sunday. The

participants who consumed alcohol within 2 days were classified as acute alcohol drinking groups. Consequently, Experiment 1 included five naturally occurring groups: abstainers, moderate drinkers with and without acute alcohol drinking, and binge drinkers with and without acute alcohol drinking. Additionally, the experiment time was always from 12pm and 6pm to minimize the effect of diurnal variation on pain and stress hormones. Another experimenter obtained an informed consent, reviewed compliance of study preparation, checked vital signs before the first blood draw, and provided debriefing after pain testing. In cases of non-compliance with the study preparation, the experiment was rescheduled. The other experimenter who was blinded to participants' drinking history drew blood and performed QST. After the QST, participants completed the questionnaires, which included first their guess about the study purpose, followed by alcohol related questionnaires (the AHS, ACQ, and recent drinking). None mentioned alcohol and all were blinded about the study hypothesis.

Before and after the blood draw, BP, HR, and body temperature were measured to make sure the levels were within normal limits. Blood was drawn in a wet-lab space. A 23g needle was used to draw 7ml of blood, which was collected in a chilled EDTA tube. Then, the blood samples were centrifuged for 15 minutes at 1000rcf in a cold room (4ºC), aliquoted into 0.5ml tubes, and stored in a -80^{0}C freezer until being assayed. Most blood samples were drawn with one attempt; however, 4.5% of the samples were drawn with two attempts at pre- and post-QST each, and 0.6% and 2.3% of the samples were drawn with three attempts at pre- and post-QST, respectively. After the blood draw, participants walked to a sensory testing room located on the same floor as the wet lab and within a 1-

minute walking distance. While moving from rooms, a water fountain, and a restroom were available. Participants were allowed to drink water and use the restroom as they desired.

In the sensory testing room, sensors were applied before the survey, and the baseline physiological data were collected for 5 minutes after the survey. The interval between different QSTs was 5 minutes. No participants withdrew their consent during or after the laboratory testing. The timeline of the experiment is depicted in Fig. 10.

<table>
<tr><th>1</th><th>2</th><th>3</th><th colspan="3">4</th><th>5</th><th>6</th><th>7</th></tr>
<tr><td rowspan="2">V/S
BAC
Blood draw</td><td rowspan="2">Survey</td><td rowspan="2"></td><td colspan="3">QST</td><td rowspan="2"></td><td rowspan="2">Survey</td><td rowspan="2">Blood draw
V/S</td></tr>
<tr><td>MPT</td><td>HPT</td><td>PPT</td></tr>
<tr><td></td><td>Physio</td><td colspan="3">Physio</td><td>Physio</td><td></td><td></td></tr>
</table>

Fig. 10. Experiment 1 timeline. [1 hour] V/S: Vital Sign (BP, HR, BT) MPT: Mechanical Pain Threshold, HPT: Heat Pain Threshold, PPT: Pressure Pain Threshold.

3.3 Statistical Analysis

The statistical assumptions were examined. Normality of each variable was evaluated with the Shapiro-Wilk's test. If violated, variables were transformed, or appropriate nonparametric tests were used. Box plot and Mahalanobis Distance tests were conducted to screen for univariate and multivariate outliers, respectively. In case of extreme outliers (3+ *SD* or $p < .001$), nonparametric tests were used to reduce a Type I or II error (45). In cases of small number of outliers, parametric tests were conducted without the outliers. Box's *M* test and Levene's test were conducted to examine homogeneity of covariance matrices for MANOVA and homoscedasticity for ANOVA, respectively. A

liberal *p* value of .01 was used for the extremely sensitive Box's *M* test (46). A correlation matrix was reviewed to evaluate multicollinearity issue (correlations > .90) for MANOVA (45).

To examine the first hypothesis, a MANOVA with three pain thresholds was conducted, followed by least significant difference (LSD = $t\sqrt{\frac{2MS_{error}}{n}}$). Although LSD dose not correct for familywise type I error, LSD was chosen because the sample size was calculated based on the large effect and the final sample did not have the sufficient number of participants in the group of interest (i.e., binge drinkers in the acute drinking phase). For the second hypothesis, an ANOVA was conducted to compare the group difference in stress hormones (i.e., epinephrine and cortisol) at baseline and their change scores (= the levels at post – baseline). If pain sensitivity would be different between the groups and the stress hormones showed parallel patterns, bias-corrected bootstrap mediational analysis would be conducted. Next, the relationship between negative affect and pain sensitivity was contrasted between the binge drinker groups and the other groups. Finally, an ANOVA was conducted to examine differences in alcohol craving between the groups. All statistical tests were two-tailed and *p* values less than .05 were considered significant, unless otherwise noted. SPSS 22 was used for all analyses except AMOS 18, which would be used for bias-corrected bootstrap analysis.

Sample Size Calculation

GPower 3.1 software was used to estimate the sample size. Large effects of alcohol withdrawal on pain threshold were reported in both animals and humans (Cohen's *d* >

1.65). (6,7,17) Therefore, an f value of .4, a large effect size (Cohen, 1988), was used. This study was designed to recruit five naturally occurring groups: abstainers, moderate drinkers with and without drinking alcohol within 48 hours, and binge drinkers with and without drinking within 48 hours prior to the experiment. Throughout this paper, alcohol consumption within 48 hours is called "acute drinking." The result of sample size calculation indicated that at least 16 participants would be needed in each group to achieve 80% power and an α of .05 for a between-subjects design. To examine stress hormones as a mediator with bias-corrected bootstrap test, 34 binge drinkers would be needed for a large effect size with 80% power (46). The adequate sample size to detect the moderating role of negative affect was not pre-determined because this moderation effect was explorative in Experiment 1. To test the three hypotheses, the required sample size was 34 participants per group. Despite efforts to meet this accrual target, recruitment of 34 moderate and 34 binge drinkers in the acute drinking phase was not successful. Additionally, Experiment 1 was planned to recruit an equal number of men and women to control for the potential gender difference and the main interest was to evaluate the group differences by drinking patterns. Consequently, all the data were analyzed and presented by the group. However, gender by group interaction was examined and significant results would be noted if any.

Missing Data

There were few missing values (1.7%) and pairwise deletion was used. Details about the missing values are as follows. For pain data, three participants did not undergo heat pain threshold tests because of Medoc malfunctioning. Because these three

participants did not experience the heat pain threshold tests, their physiological and psychological state data during and after the heat pain test were not analyzed. Psychological trait variables had no missing values. To avoid missing by mistake, a popup window showed up on the screen prompting the participant to respond to unanswered items or to continue without answering. For psychological state variables, one abstainer and one moderate drinker did not complete the SAM-valence, arousal, and dominance throughout the experiment and one binge drinker did not complete the STAI at baseline.

4. RESULTS

EXPERIMENT 1

Of 179 consented participants, 177 participants completed this experiment. Two did not undergo the QST due to hypotension (systolic blood pressure was 80) at baseline and vasovagal syncope after the first blood draw. Demographics are shown in Table 1. There were no differences in gender, ethnicity, or cigarette use between the groups (*p*s > .097). The abstainer group was about one year younger than the other groups, but all were young adults. The typical number of drinks was on average 2-3 drinks for moderate drinkers and 4 (female)/6-8 (male) drinks for binge drinkers (Table 1).

Table 1. Experiment 1 Demographics and drinking patterns

	Abstainers	Moderate drinkers		Binge drinkers		
		No acute Drinking	Acute drinking	No acute Drinking	Acute drinking	
	M(*SD*)	*M*(*SD*)	*M*(*SD*)	*M*(*SD*)	*M*(*SD*)	*F*
N	43	50	23	36	25	
(Male/Female)	(20/23)	(25/25)	(17/6)	(15/21)	(14/11)	6.69
% Caucasian	60.5	64.0	73.9	63.9	80.0	3.57
% Cigarette use	2.3	6.0	13.0	13.9	8.0	
Age	18.7(1.2)^{a}	20.1(2.5)^{b}	20.3(2.0)^{b}	19.8(1.8)^{b}	19.6(2.3)^{ab}	4.35*
Typical drinks						
Men	-	1.7(1.5)	3.3(2.3)	7.6(7.2)	5.5(2.9)	
Women	-	2.3(1.1)	1.6(1.0)	4.2(2.3)	4.1(1.6)	
Ages at first drink	-	18.0(1.9)^{a}	16.8(1.9)^{b}	17.1(1.3)^{b}	16.5(1.0)^{b}	4.87*
Years of drink	-	2.2(2.1)	2.8(1.9)	2.6(1.9)	3.0(2.3)	15.67
Frequency	-	2.0(1.4)	2.0(1.6)	2.6(1.5)	2.2(2.4)	0.82
HSS	-	3.0(2.8)^{a}	4.0(3.0)^{a}	5.1(3.0)^{b}	6.6(2.9)^{c}	
AHS$^{\chi}$	-	5.6(3.7)^{a}	5.6(3.0)^{a}	6.3(4.0)^{a}	10.2(9.3)^{b}	4.94*

Acute drinking: alcohol consumption within 48 hours before the experiment; * $p < .005$, χ^2 statistics were used for gender and ethnicity; superscripts of *a* and *b* indicate significant difference in post hoc analysis; AHS: Acute Hangover Scale.

The age of the first alcohol use was somewhat later in moderate drinkers than the others, but no significant difference was found in years of regular alcohol use (p = .448). On the DDQ prescreening questionnaire, frequency of drinking for the past month was evaluated. The survey has a nominal scale, ranging from 0: *not drink at all* to 7: *once a day or more*. The results of an ANOVA indicated no significant difference in the frequency. All types of drinkers endorsed drinking alcohol on average about twice a month. However, binge drinkers reported more experiences of hangover symptoms for the past 12 month on the HSS in their prescreening survey. Notably, the HSS scores were even higher in the binge drinkers with acute drinking than those without. As expected, more acute hangover symptoms were reported by binge drinkers with acute drinking, validating the self-reported recent drinking episode.

4.1 Psychological Traits

Psychological traits were only significantly different between the groups for depressive symptoms. The results of the Kruskal-Wallis test indicated a significant difference in depressive symptoms (p = .022), but not in anxiety symptoms (p =.166) or stress levels (p = .671). Post-hoc pairwise comparison with the Mann-Whitney test showed that moderate drinker and binge drinker groups reported higher levels of depression than the abstainer group (Table 2). Depressive symptoms were not different between the abstainer and moderate drinker group with acute drinking (p = .075).

The result of an ANOVA indicated the total PSS scores also did not differ between the groups (p = .329), suggesting perceived levels of stress were similar between the groups with different drinking patterns. In the 2 (gender) by 5 (group) ANOVA, there was

a significant gender difference, F (1, 167) = 4.58, p = .034, η^2 = .027 with women (M = 17.8, SD = 6.0) reporting higher levels of stress than men (M = 15.5, SD = 5.7). No gender by group interaction was observed (p = .401).

Table 2. Experiment 1 Psychological traits

	Abstainers		Moderate drinkers				Binge drinkers			
			No Acute Drinking		Acute drinking		No Acute Drinking		Acute drinking	
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
DASS-DEP	3.8^{a}	5.2	6.2^{b}	7.2	5.9^{ab}	6.5	5.3^{b}	5.1	7.9^{b}	7.3
DASS-ANX	4.1	4.5	4.8	5.0	3.7	3.7	4.6	5.1	6.3	4.5
DASS-STR	8.9	6.0	8.9	6.3	8.6	6.6	9.3	6.6	11.0	6.7
PSS	15.5	5.7	16.3	6.1	16.1	5.9	17.9	5.5	17.9	6.8
BIS-Total*	4.1	0.2	4.1	0.2	4.1	0.1	4.1	0.2	4.2	0.2
BIS-ATTEN*	2.8	0.2	2.8	0.2	2.9	0.2	2.8	0.2	2.8	0.3
BIS-MOTOR*	3.0	0.2	3.0	0.2	3.1	0.2	3.1	0.2	3.1	0.2
BIS-PLAN*	3.1	0.2	3.2	0.2	3.1	0.2	3.2	0.2	3.2	0.2
ACQ-Total*	3.0^{a}	0.3	3.2^{b}	0.3	$3.3^{b,c}$	0.3	$3.3^{b,c}$	0.4	3.6^{c}	0.3
ACQ-COMP	3.5	1.6	3.7	1.6	4.6	2.8	4.0	2.0	4.5	2.3
ACQ-XPCT	4.5^{a}	2.0	6.9^{b}	3.5	6.8^{b}	3.3	7.6^{b}	3.8	8.0^{b}	3.3
ACQ-PURP	6.9^{a}	3.8	9.0^{b}	3.9	10.4^{b}	4.0	10.4^{b}	4.6	10.0^{b}	3.4
ACQ-EMOT	5.5	3.2	5.6	3.6	5.8	3.4	6.3	3.8	7.5	4.5

DASS (Depression Anxiety Stress Scales)-DEP: Depression, ANX: Anxiety, STR: Stress; PSS: Perceived Stress Scale; BIS (Barratt Impulsiveness Scale)-ATTEN: Inattention, MOTOR: Motor Restlessness, PLAN: Non-Planning; ACQ (Acute Craving Questionnaire-NOW)-COMP: Compulsivity; XPCT: Expectancy; PURP: Purposefulness; EMOT: Emotionality; superscripts of *a*, *b* and *c* indicate significant difference in post hoc analysis. * log transformed.

The result of an ANOVA indicated that BIS scores were not significantly different between the groups (p = .315, Table 2). Therefore, the levels of impulsivity were similar between the groups with different drinking patterns. When analyzing the three subscale

scores with a MANOVA, the levels of inattention, motor restlessness and non-planning were similar between the groups (p = .556).

Finally, the result of an ANOVA with LnACQ total scores showed a group difference in alcohol craving after the pain testing, F (4, 169) = 7.19, p < .001, Table 2. The results of post hoc analysis with LSD showed that alcohol craving was greater in moderate and binge drinker groups than the abstainer group. Furthermore, the binge drinker group with an acute drinking episode showed greater craving than the moderate drinker group without acute alcohol drinking. Consequently, acute alcohol withdrawal might further enhance craving for alcohol.

Additional analysis with the four ACQ subscale scores (compulsivity, expectancy, purposefulness, and emotionality) were conducted to further examine whether the group difference in overall craving was related to specific aspects of craving. After removing the three multivariate outliers (1 abstainer, 2 binge drinkers), a MANOVA was conducted. The results indicated a main effect of group, Wilk's Λ = .78, p < .001. The follow-up univariate analysis showed that only expectancy, F (4, 169) = 6.95, p < .001, partial η^2 = .14, and purposefulness, F (4, 169) = 4.82, p = .001, partial η^2 = .10, were different between the groups. LSD post-hoc analysis indicated that expectancy and purposefulness scores were higher in the moderate- and binge-drinker groups than the abstainer group. Acute drinking episode did not further enhance craving for both the moderate and binge drinker groups evidenced by no significant differences between the drinking groups with and without acute drinking, ps > .627. Taken together, the data showed differences between abstainers and drinkers in urges and desires to drink alcohol in expecting the

positive aspects of drinking and with intentions or plans to drink, but there was no difference between moderate and binge drinkers.

Table 3. Experiment 1 Baseline psychological and physiological states

	Abstainers		Moderate drinkers				Binge drinkers			
			No Acute Drinking		Acute Drinking		No Acute Drinking		Acute Drinking	
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
Psychological State										
STAI	9.2	2.3	9.2	2.3	9.4	2.5	9.4	3.1	9.4	2.6
PANAS-PA	31.7	6.7	30.9	6.5	31.9	8.9	29.2	7.7	30.0	6.9
PANAS-NA	12.8	3.2	12.9	3.1	12.4	1.8	12.4	2.5	14.0	4.2
SAM-V	6.6	1.6	6.4	1.3	6.0	1.8	6.6	1.5	5.9	1.2
SAM-A	3.3	1.9	3.1	1.8	3.3	2.1	3.4	2.1	3.7	1.4
SAM-D	5.4	1.9	4.8	1.6	5.5	2.0	5.4	1.9	5.3	1.6
Physiological State										
HR(beat/min)	72.7	8.9	75.6	10.7	72.0	9.5	74.3	10.1	72.8	9.5
sqrtSCL(μS)	2.0^{a}	0.7	2.5^{b}	0.8	2.5^{b}	0.6	2.1^{ab}	0.6	2.4^{b}	0.8
RR(breath/min)	13.6	3.9	14.2	2.8	14.1	3.3	13.2	3.2	13.9	3.0
SBP(mmHg)*	112.0	10.0	114.5	13.1	119.4	13.7	113.9	12.7	116.8	12.9
DBP(mmHg)*	70.6	5.7	71.8	7.4	72.7	10.2	72.1	9.0	71.0	6.5

STAI: State-Trait Anxiety Inventory-6, PANAS (Positive and Negative Affect Schedule)-PA: Positive affect, NA- Negative affect, SAM (Self-Assessment Manikin)-V: Valence, A-Arousal, D: Dominance; HR: Heart Rate, SCL: Skin Conductance Level, RR: Respiration; SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure;*: Kruskal-Wallis followed by Mann-Whitney *U*.

4.2 Baseline Psychological and Physiological States

All baseline psychological states were similar across groups as indicated by non-significant results of Kruskal-Wallis tests (*p*s > .136). For the baseline physiological states, a one-way ANOVA indicated difference in sqrtSCLs, $F(4, 283) = 3.12$, $p = .017$, partial $\eta^2 = .07$. LSD post-hoc analysis indicated that higher SCLs were observed in the two moderate drinker groups and the binge drinker group with acute drinking, *p*s < .05 (Table

3). No significant group difference was found in HR, F (4, 172) = 0.86, p = .488, and RR, F (4, 172) = 0.56, p = .692. The Kruskal-Wallis tests were conducted to compare blood pressures (systolic and diastolic). Systolic (SBP) and diagnostic blood pressure (DBP) were not different between the groups (ps > .310).

In controlling for ethnicity (White = 1 vs other ethnicity = 0), group difference in SCLs remained significant (p = .018). However, LSD post hoc analyses showed that higher levels of SCL were observed only in the two moderate drinker groups, not in the binge drinker group with acute alcohol use.

4.3 Pain Sensitivity

To examine the group difference in mechanical, heat, and pressure pain thresholds, a MANOVA was conducted. The result indicated a main effect of group, Wilk's Λ = .80, p < .001. The result of the follow-up univariate analysis indicated that only pressure pain threshold (log transformed) was different between the groups, F(4, 165) = 7.10, p < .001, and this was a large effect (partial η^2 = .14). No significant group difference was observed in mechanical pain threshold (log transformed, p = .148) or heat pain threshold (p = .393). LSD post-hoc analysis indicated that the binge drinker group without acute drinking, M = 1.02, SD = 0.50, showed reduced pressure pain thresholds when compared to the moderate drinking group without acute drinking, M = 1.27, SD = 0.47, p = .013, Fig. 11. These results indicate the divergent effects of alcohol consumption on pressure pain threshold. Specifically, moderate drinking was associated with a slight increase in pressure pain threshold whereas binge drinking was associated with a slight decrease in pressure pain threshold. Additionally, the binge drinker group with acute drinking, M = 0.67, SD = 0.51,

showed reduced pressure pain threshold compared to all the other groups, *p*s < .005. The difference between the binge drinker groups with and without acute drinking indicated that acute alcohol withdrawal was associated with further reduction in pressure pain thresholds. Lastly, the result indicated that binge drinking and acute alcohol withdrawal might not influence cutaneous mechanical and heat pain threshold in young adult binge drinkers.

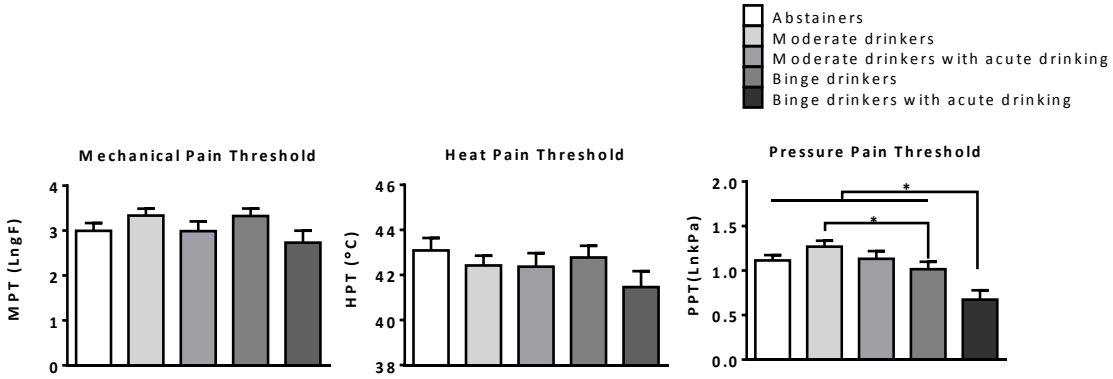

Fig. 11. Comparison of mechanical, heat, and pressure pain thresholds between the groups with different drinking patterns. Error bars = SEM.

4.4 The Role of Stress Hormones in Pain Sensitivity

First, the group differences in the baseline epinephrine levels were examined (Fig. 12, left). Because of gender difference in epinephrine levels, a 2 (gender) by 5 (group) ANOVA was conducted. The result indicated a significant main effect of group, $F(4, 167) = 2.50$ $p = .044$, partial $\eta^2 = .06$, and gender, $F(4, 167) = 7.89$, $p = .006$, partial $\eta^2 = .05$, but no significant interaction ($p = .938$). For a significant main effect of gender, the basal

epinephrine levels were higher in the men (M = 247.4, SD = 202.5) than the women (M = 164.5, SD = 151.0).

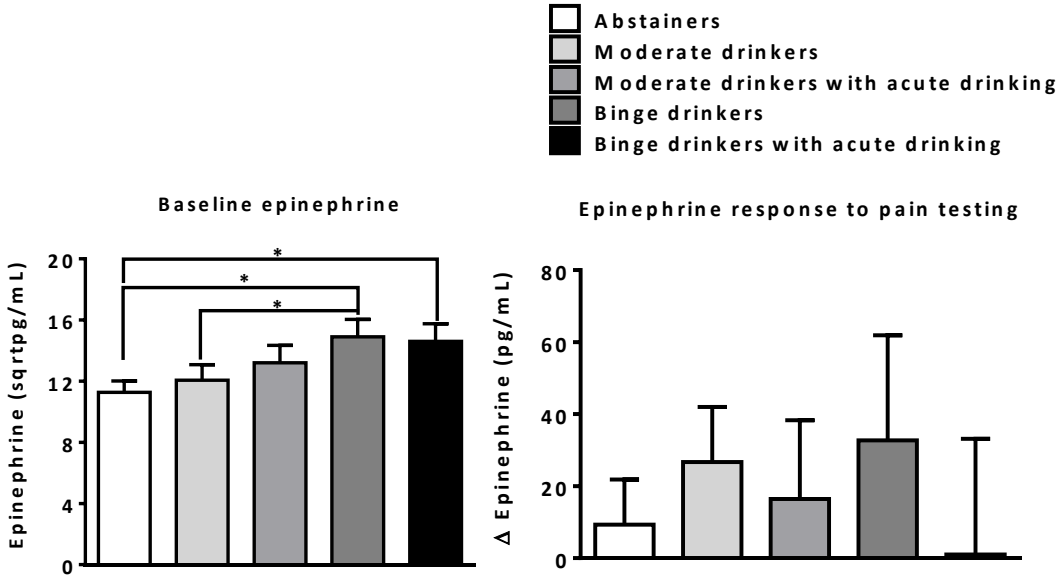

Fig. 12. Comparison of baseline epinephrine levels and their responses to pain between the groups with different drinking patterns. Error bars = SEM.

LSD post-hoc analysis showed that regardless of acute drinking, the two binge drinker groups showed elevated epinephrine at baseline compared to the abstainer group. Additionally, the binge drinker group without acute alcohol drinking showed elevated epinephrine levels compared to the moderate drinker group without acute alcohol drinking. Different from expectation, no significant difference was found between the binge drinkers with and without acute drinking. These results suggested that binge drinking was associated with increases in basal epinephrine levels, but acute alcohol withdrawal was not associated with further increase in epinephrine levels. This

epinephrine pattern did not completely corresponding to the pattern of pressure pain thresholds. In particular, the binge drinker group with acute alcohol drinking did not show the highest elevation and only differed from the abstainer group. Therefore, a bias-corrected bootstrap test was not performed. However, binge drinkers showed higher epinephrine levels than the moderate drinkers when they had not drunk alcohol within 48 hours. Another similarity was that the binge drinker group with acute drinking showed higher levels of epinephrine than the abstainer group. This suggests that basal epinephrine levels may contribute to binge drinking-induced hypersensitivity to pressure pain. Subsequently, an ANCOVA with basal epinephrine levels as a covariate was conducted. In controlling for basal epinephrine levels, the group difference in pressure pain threshold remained significant, F (4, 171) = 6.21, $p < .001$, but the effect size was reduced by 7%, partial $\eta^2 = .13$. This suggests that epinephrine did not fully account for the group difference in pressure pain thresholds, but it explained a small portion of the variance (7%).

The Kruskal-Wallis test was conducted to examine whether epinephrine responses to laboratory pain testing were significantly different between the groups (Fig. 12, right). This analysis used the change score (= the levels at post-pain testing– the baseline). The result indicated no significant group difference (ps = .727). Overall, epinephrine levels were elevated after the QSTs (M = 0.6, SD = 3.8).

Next, the group difference in the baseline cortisol levels was examined (Fig. 13, left). Because of gender difference in cortisol levels, a 2 (gender) by 5 (group) ANOVA was conducted. The result indicated a significant main effect of gender, F (1, 167) = 4.19,

p = .040, η^2 = .02, but no significant main effect of group (p = .215) or group by gender interaction (p = .402). Similarly to the epinephrine pattern, the cortisol levels were higher in the men (M = 123.2, SD = 45.1) than the women (M = 105.8, SD = 38.2). The lack of a group difference in cortisol suggests that basal cortisol did not contribute to the group difference in pressure pain threshold.

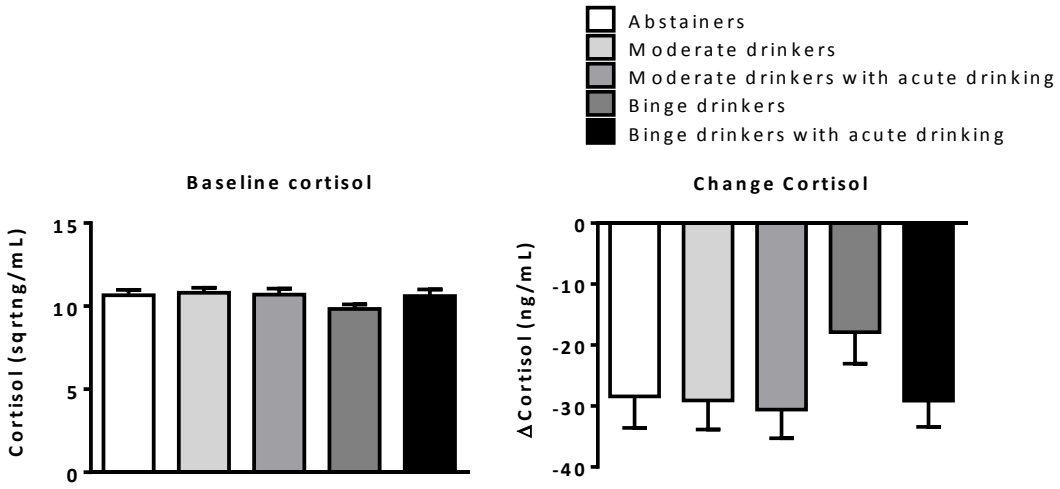

Fig. 13. Comparison of baseline cortisol levels and their responses to pain between the groups with different drinking patterns. Error bars = SEM.

The Kruskal-Wallis test was conducted to examine whether cortisol responses to laboratory pain testing were significantly different between the groups (Fig. 13, right). The result indicated no significant group difference (p = .435). On average, cortisol levels were decreased after the QSTs (M = -26.8, SD = 30.5). This cortisol response to pain testing is different from epinephrine response; the former showed reduction and the latter

showed elevation at post-pain testing. The baseline levels of epinephrine and cortisol were unrelated (Pearson r = -.06, p = .446).

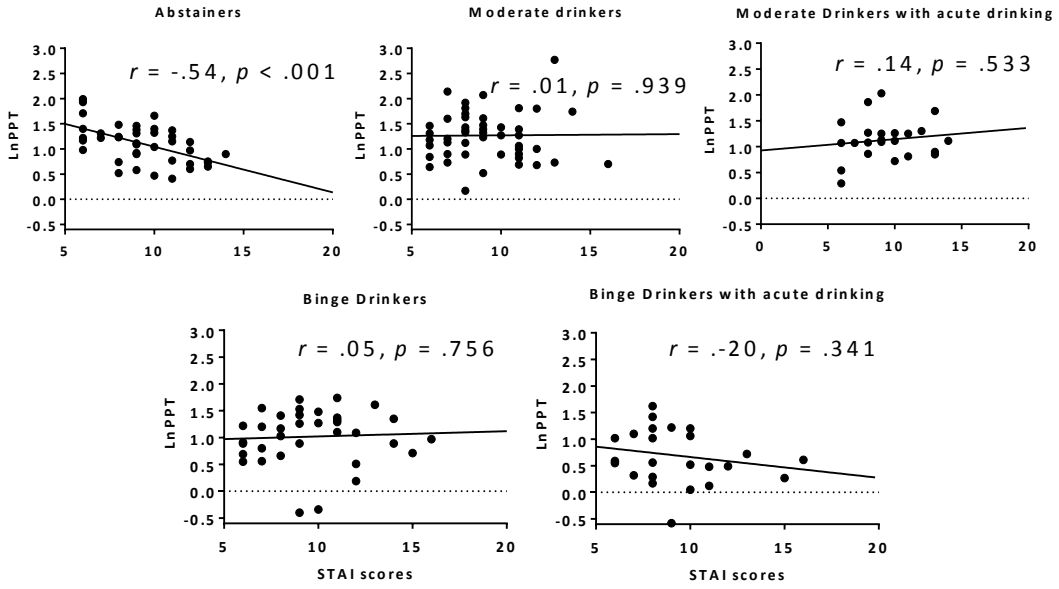

Fig. 14. Pearson correlations between baseline STAI scores and PPT (pressure pain threshold) were compared across the groups with different drinking patterns.

4.5 The Role of Negative Affect in Pain Sensitivity

The Pearson correlation coefficients between state anxiety and pain sensitivity were compared between the binge drinker and the abstainer groups (Fig. 14). A significant relationship was found only in the abstainer group, with higher levels of anxiety at baseline as being associated with lower pressure pain thresholds (r = -.54, p < .001). The same pattern was observed with other measures of pain sensitivity, with the abstainer group reporting greater anxiety at baseline associated with lower mechanical (r = -.37, p = .013) and heat pain thresholds (r = -.34, p = .027). Different from our expectation, this result

showed binge drinking and acute withdrawal eliminated the relationship between baseline negative affect and pain sensitivity. To note, changes in anxiety levels by the three QSTs was minimal (M = 0.07, SD = 2.42) and the Kruskal-Wallis test indicated no group difference (p = .732). Psychological traits (depression, anxiety, and stress levels) and their interactions with the group did not predict mechanical, heat, and pressure pain thresholds (ps > .101).

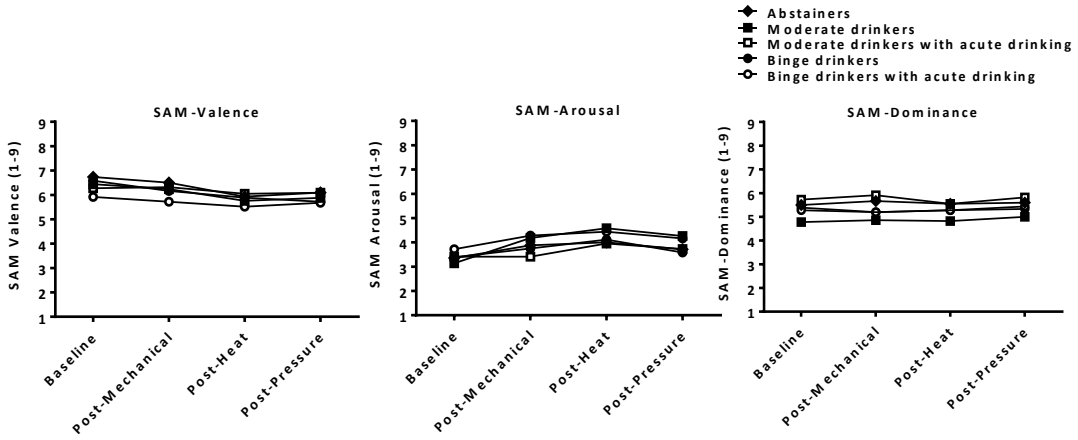

Fig 15. Changes in the SAM-valence, arousal, and dominance in response to pain tests

4.6 Psychological Responses to Pain Testing

First, changes in SAM-valence scores were compared between the groups, Fig. 15, the top. The result of a 4 (time) by 5 (group) repeated measures ANOVA showed a significant change over time, F (3, 438) = 21.89, p < .001, Greenhouse-Geisser ε = .86 without significant interaction (p = .311). A linear function best fit the data, suggesting that unpleasant emotion decreased over time, F (1, 170) = 38.25, p < .001.

With the SAM-arousal scores, a 4 (time) by 5 (group) repeated measures ANOVA was conducted. The result showed a significant change over time, F (2, 415) = 17.07, p < .001, Greenhouse-Geisser ε = .81 without significant interaction (p = .135). A quadratic function best fist the data, F (1, 170) = 28.40, p < .001. LSD post-hoc analysis showed that the levels of perceived arousal increased up to the second pain testing, but decreased upon completion of the last pain testing.

Lastly, the result of a 2 (time) by 5 (group) repeated measures ANOVA showed no significant change in the SAM-dominance scores, F (2, 423) = 1.30, p = .274, Greenhouse-Geisser ε = .83, and no significant interaction (p = .787).

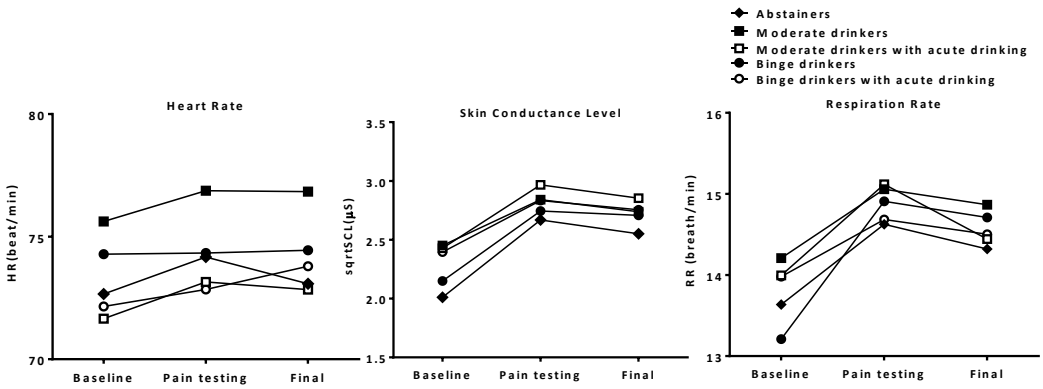

Fig. 16. Changes in HR, SCL, and RR before, during, and after pain testing

4.7 Physiological Responses to Pain Testing

Changes in HR were compared between the groups, Fig. 16, top. A 3 (time: baseline, pain testing, final) by 5 (group) repeated measures ANOVA was conducted. Because HR pattern was unchanged across different pain tests, the average HR for the

entire duration of pain tests were used. The result indicated a significant main effect of time, F (4, 742) = 5.48, p < .001, Greenhouse-Geisser ε = .74, but no interaction (p = .425). LSD post hoc analysis showed that HR was slightly elevated during pain testing (M = 74.7, SD = 9.5) and remained elevated for the 5 minutes after the pain testing (M = 74.5, SD = 9.7) compared to the baseline (M = 73.6, SD = 9.8).

Changes in SCL were compared between the groups, Fig. 16, middle. A 3 (time: baseline, pain testing, final) by 5 (group) repeated measures ANOVA was conducted. Again, the SCL pattern was unchanged across different pain tests. Therefore, the average SCL for the entire duration of pain tests were used. The result indicated a main effect of time, F (1, 214) = 212.6, p < .001, Greenhouse-Geisser ε = .63 and significant interaction, F (5, 214) = 3.17, p = .009. LSD post hoc analysis showed that SCL was elevated during pain testing (M = 2.8, SD = 0.6) compared to the baseline (M = 2.3, SD = 0.7), but it was slightly recovered after the pain testing (M = 2.7, SD = 0.6) as indicated that the level at the final was different from the levels at baseline (p < .001) and during pain testing (p < .001). Another post-hoc test for the interaction was conducted. The results indicated that initially different SCLs were no longer different during, F (4, 170) = 1.05, p = .382, and after pain testing, F (4, 170) = 0.99, p = .414. SCR to mechanical, heat, and pressure pain thresholds were also compared between the groups. The results of ANOVA showed no significant group difference (ps > .231).

Changes in RR were compared between the groups, Fig. 16 bottom. A 3 (time: baseline, pain testing, final) by 5 (group) repeated measures ANOVA was conducted. Because the RR pattern was unchanged across different pain tests, the average RR for the

entire duration of pain tests was used. The result indicated a main effect of time, F (2, 309) = 22.70, $p < .001$, Greenhouse-Geisser $\varepsilon = .91$, but no interaction ($p = .472$). LSD post hoc analysis showed that similar to SCL, RR was elevated during pain testing ($M = 14.9$, $SD = 2.3$) compared to the baseline ($M = 13.8$, $SD = 3.3$). Additionally, RR was slightly recovered after the pain testing ($M = 14.6$, $SD = 2.8$) as indicated that the rate at the final was different from the levels at baseline ($p < .001$) and during pain testing ($p < .001$).

To compare SBP changes between the groups, a 4 (time: baseline, post-mechanical pain thresholds, post-heat pain threshold test, final) by 5 (group) repeated measures ANOVA was conducted. The results showed a main effect of time, F (3, 504) = 5.28, p = .001, but no interaction ($p = .572$). LSD post hoc analysis showed SBP reduced after heat ($M = 113.0$, $SD = 12.2$, $p = .005$) and pressure pain threshold tests ($M = 112.4$, $SD = 11.9$, $p = .001$) compared to the baseline ($M = 115.0$, $SD = 11.7$).

Changes in DBP were also compared between the groups. The result of a 4 (time: baseline, post-mechanical pain threshold, post-heat pain threshold, final) by 5 (group) repeated measures ANOVA showed no main effect of time ($p = .090$). A main effect of group was found, F (3, 504) = 5.28, $p = .001$, but the results of LSD post hoc analysis were not significant (ps > .688). Throughout the experiment, DBP levels remained unchanged.

5. EXPERIMENT 2

Experiment 2 was a within-subject design to examine alcohol-withdrawal induced hyperalgesia in young adult binge drinkers with laboratory alcohol administration. Experiment 2 included mechanical, heat, and pressure pain thresholds to test replicability of Experiment 1 findings and added capsaicin-induced pain responses such as spontaneous burning pain on the site of application, primary and secondary hyperalgesia, and flare. To administer alcohol in the laboratory, Experiment 2 raised the minimum age to 21 years old. Additionally, Experiment 2 recruited students (undergraduates and graduates) and employees using university-listserv emailing method only. All the other eligibility criteria were the same. To avoid redundancy, only measures and procedures that are different from Experiment 1 are described below.

The four specific objectives for Experiment 2 were as follows. The first objective was to examine whether alcohol withdrawal would enhance spontaneous pain, primary and secondary hyperalgesia, and capsaicin-induced flare. The second objective was to examine whether stress hormones (i.e., epinephrine and cortisol) would be correlated with alcohol withdrawal-induced hyperalgesia and flare. Third, this study examined whether negative affect (i.e., depression and anxiety) would enhance alcohol withdrawal-induced hyperalgesia. The last objective was to examine whether pain and alcohol-withdrawal would enhance alcohol craving.

It was hypothesized that binge drinkers would show enhanced capsaicin-induced pain and this pain hypersensitivity would be further enhanced during acute alcohol withdrawal. Additionally, stress hormones were expected to be increased in binge drinkers

at baseline and be further enhanced during acute alcohol withdrawal. Next, more negative affect during alcohol withdrawal would enhance withdrawal-induced hyperalgesia. Lastly, alcohol withdrawal and pain would increase alcohol craving.

A certificate of confidentiality was obtained from the NIAAA to protect the privacy of participants. Texas A&M University Institutional Review Board approved the study procedures and informed consent was obtained from all participants.

Participants were recruited from June of 2015 to April of 2016. Of the total 657 women and 565 men who completed the online prescreening survey (M_{age} = 23.1, SD_{age} = 2.3, 21-30 years), 5 people did not provide a response for their current drinking pattern. Of the total 1,217 responders, 6% endorsed being former drinkers and 79% endorsed being current drinkers. This differs from Experiment 1, where abstinence was the most common (57%), heavy drinking (34%) was the most common in Experiment 2, followed by moderate (24%) and binge drinking (21%), Fig. 17. About a fourth of individuals who completed the prescreening were not invited due to ineligibility criteria.

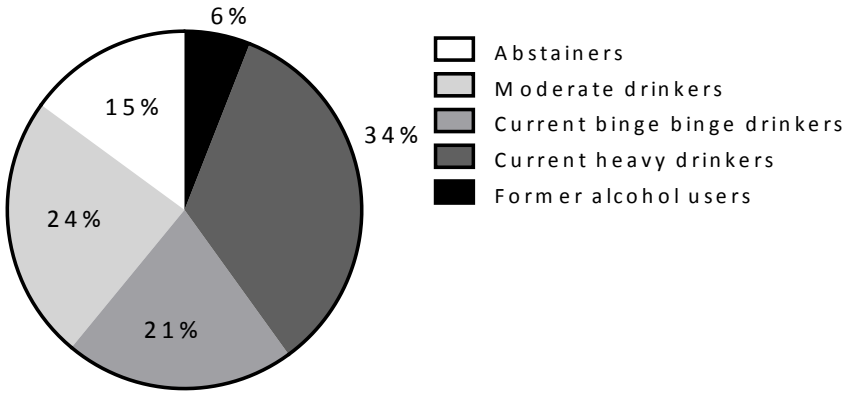

Fig. 17. Experiment 1 prevalence of different drinking patterns in young adults

Of the total 946 eligible participants who were invited via email, 54 people were consented and 41 participants completed the study. Of those who completed the study, five people were excluded because the exit interview with the SCID-I revealed that they underreported their current drinking on the prescreening survey. These five people were replaced and recruitment was continued until the study had 12 each of abstainers, moderate drinkers, and binge drinkers. Although the study intended to recruit equal number of men and women for all drinking pattern, abstainers consisted of five females and seven males because miscoded gender on the recruitment tracker was found after recruitment was completed. Of the 13 participants who were consented, two did not undergo QST because their ineligibility condition was found after consenting. One had skin condition on the pain testing sites and the other reported pain from sprained toes. Three participants were removed by the experimenter after the QST was completed on the first visit because of the following reasons. One participant experienced vasovagal syncope at the blood draw after the QST. Another participant endorsed drinking within two days after the QST. Lastly, one participant who expressed extreme worry over blood tests was removed from the study. The rest (n = 9) completed their first study visit, but withdrew their consent or did not show up for the subsequent visits. Most people who drank juice or alcohol on the second visit showed up for their last study visit except two female binge drinkers who consumed alcohol on the second visit, but did not show up on the next day.

When using the DSM-IV diagnostic criteria, 33.3% of binge drinkers met the current diagnostic criteria for alcohol abuse and 25.0% met the diagnostic criteria for alcohol dependence. Some moderate drinkers also met the current (16.7%) and lifetime

(8.3%) diagnostic criteria for alcohol abuse. Two moderate drinkers did not have a current problem, but met lifetime diagnostic criteria for alcohol dependence. In using the DSM-5 diagnostic criteria, 75% (58.3% mild, 8.3% moderate, and 8.3% severe) of binge drinkers met the diagnostic for alcohol use disorder. Additionally, 23.1% of moderate drinkers met the diagnostic criteria for mild alcohol use disorder. Using the AUDIT (the total score 8+), 3 moderate drinkers (25%) and 5 binge drinkers (42%) were identified as having problematic drinking behaviors.

6. METHODS

EXPERIMENT 2

6.1 Measures

In addition to the measures used in Experiment 1, the following psychological trait measures were added in Experiment 2.

Self-report

The **UPPS Impulsive Behavior Scale** (UPPS) is a 45-item self-report questionnaire to assess four dimensions of impulsivity such as lack of premeditation, urgency, sensation seeking, and lack of perseverance. The scale ranges from 1: *agree strongly* to 4: *disagree strongly* and higher total summed scores indicates more impulsive behavior (Cronbach's αs =.83 - .89) (47).

The **Zuckerman-Kuhlman Personality Questionnaire**–Impulsive Sensation Seeking subscale (ZKPQ-ImpSS) is a true/false questionnaire to assess impulsivity with 19 items (Cronbach's αs =.64 - .80) (48,49). This subscale measure assesses a lack of planning and a tendency to act impulsively and to seek excitement and novel experiences. Higher scores indicate higher levels of impulsivity and sensation seeking tendency.

The **Biphasic Alcohol Effects Scale** (BAES) is a self-report to measure of the subjective acute stimulant (7 items, Cronbach's α = .94) and sedative effects (7 items, Cronbach's α = .85) of alcohol (50). The scale ranges from 0: *not at all* to 10: *extremely*. The possible total summed scores range from 0 to 70 for each effect. The BAES was administered on the last study to assess the effect of consumed alcohol in the laboratory on the previous night.

The **Alcohol Use Disorders Identification Test** (AUDIT) is a measure widely used measure to detect individuals with problematic drinking (Cronbach's $\alpha = .81$) (51). This 10-item measure has acceptable sensitivity (.81), specificity (.95), and diagnostic accuracy (AUC = .97) (51).A cutoff score of 8 has been suggested to maximize sensitivity to the mid .09s (52). This measure also has acceptable sensitivity (.84) and specificity (.71) for college students (53). This measure was administered on the last study visit.

The **Structural Clinical Interview for DSM-IV Axis I Disorder** (SCID-I)-Alcohol Abuse and Dependence module is a semi-structured interview for the diagnosis of alcohol abuse and dependence for the lifetime alcohol use problems. The new DSM-5 diagnostic criteria exclude a prior legal problem item and include a new item assessing focused attention on alcohol use (10). The DSM-5 does not use the term alcohol abuse and dependence, but instead uses a new term, alcohol use disorder with different severity (mild, moderate, and severe).

Quantitative Sensory Testing (QST)

Pain testing was conducted on the dorsum of the feet. To measure changes in mechanical pain sensitivity before and after topical capsaicin on the application site and the non-application site, a grid was drawn on both feet before the QSTs (Fig. 18, left). The middle of the foot was marked with a 1.6cm diameter circle for the capsaicin application site and eight spokes of a 5cm length line were drawn at 45º angle. On each spoke, 10 dots spaced 0.5cm apart were marked with a washable marker for evaluating pain intensity with a 26gF von Fey filament. To draw the grid on the anatomically same location on the dominant foot, the second toe was used as an index for the central line. The von Frey was

applied first to the non-dominant foot, starting from the furthest point and moving towards the capsaicin application site. The first test always started from the second toe, then moved to the opposite spoke to avoid local influence, and moved to the outer side (toward the 5th phalange, Fig. 18, left).

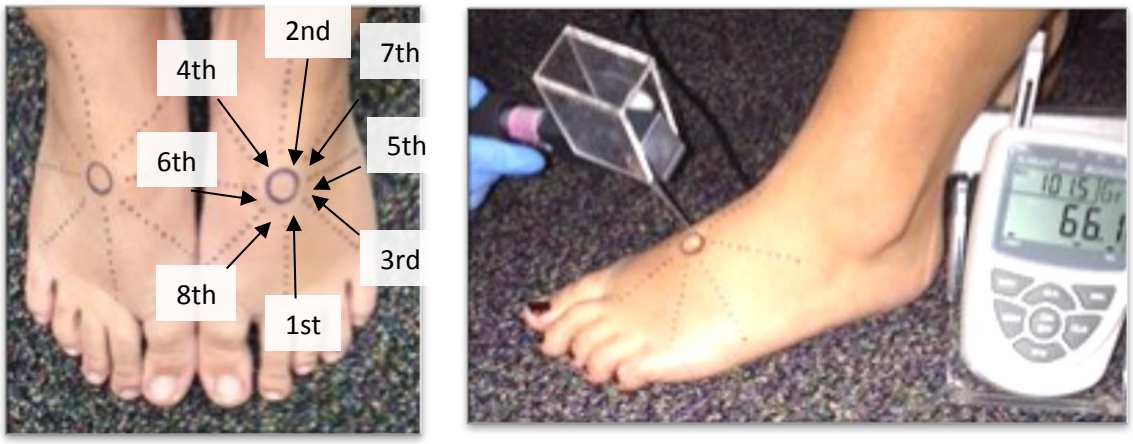

Fig. 18. The grids on the pain testing sites (left) and electronic von Frey application (right)

Participants marked the pain intensity after each von Frey application on paper using a VAS. For the mechanical pain thresholds, an electronic von Frey anesthesiometer with semi-flexible tip (Almemo 2450, IITC Inc, Life Science, Wooland Hill, CA) was applied to the capsaicin application site (non-dominant foot) and the counterpart (dominant foot), Fig. 18, right. Participants were instructed to say "NOW" when they first felt a sharp pain sensation. Three mechanical pain threshold tests were conducted at 10s intervals. The average of the three was used for the analysis. The protocols for assessing

heat and pressure pain thresholds were the same as Experiment 1 and tested on the non-dominant foot.

Capsaicin Application First, 0.3ml of 6% capsaicin solution dissolved in a 50% ethanol (Cat No. 360376-1G, Sigma Aldrich Inc,) was placed on a 1.5cm diameter filter paper. Then, the capsaicin-containing filter paper was applied for 30 minutes to the center of the non-dominant foot and the site was covered with 6cm × 7cm Tegaderm (3M, St. Paul Minnesota, USA, Fig. 19). The vehicle, 0.3ml of 50% ethanol solution applied to the same size filter paper, was applied to the dominant foot. About 16ml of 6% capsaicin solution was premade every 6 month and stored at a 4ºC in a refrigerator. The solution was kept on a room temperature for about 30 minutes before use.

Fig. 19. Capsaicin application on a filter paper

Visual Analogue Scale (VAS) A paper version of the visual analogue scale (VAS) was used to evaluate sensory and affective pain intensity. The VAS is a 10cm horizontal line, anchored by words and numbers at each end: 0: *not at all painful* and 10: *the most*

intense pain imaginable for sensory pain intensity and 0: *not at all unpleasant* and 10: *the most unpleasantness imaginable* for affective pain intensity (54). Distance from the zero measured in cm to one decimal point was used for intensity.

Primary Allodynia Mechanical pain thresholds were measured three times on the capsaicin and vehicle solution application site before and after the solution application. Primary hyperalgesia was measured by calculating the difference score before and after the capsaicin application. Changes in pain threshold would reflect sensitization or desensitization.

Secondary Allodynia Area of secondary allodynia was determined with two methods. The first method was to calculate the size of the area where a persistent increase in pain intensity occurred compared to the furthest point on each spoke. This method assumes pain sensitivity on the entire dorsal surface of the foot is the same. The total possible values range from 1.8 (the size of the application) to 93.8cm^2. The second method was to calculate the size of the area where pain intensity increased compared to the baseline. This method assumes that pain sensitivity on the surface is not the same so that direct point-by-point comparisons should be made before and after the capsaicin application. The possible total values range from 1.8 to 111.5cm^2. These two methods were also used to calculate areas of hyperalgesia on the vehicle foot to evaluate central sensitization.

Laser Doppler Imaging A laser Doppler imager (MoorLDI2-IR model, Moore Instruments Ltd., London, UK) was used to assess capsaicin-induced neurogenic flare reaction. This LDI scan (256×256 pixels; spatial resolution of 4ms/pixel) was placed at a

distance of 50cm from the skin. The scan was performed immediately after the 30 minutes of capsaicin application and took about 5 minutes per image. The scanned image was analyzed offline using the Moore LDI image review 5.3 software to calculate size (cm^2) and intensity (arbitrary perfusion units: PU) of the erythematous flare reactions. The size of flare was calculated for the zones of hyperemic responses (> 300 PU) (55,56). The participants were asked to lay on a table in a supine position, to keep their foot flat, and to stay still for 5 minutes. For those who were unable to stay still, the experimenter held their knee with permission (Fig. 20).

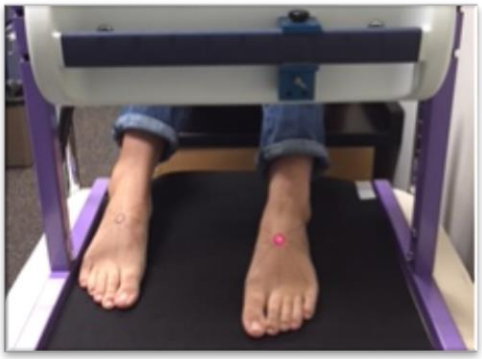

Fig. 20. Laser Doppler imaging to measure flare in a supine position

Physiological Measures Collection, cleaning, and analysis method for physiological data were the same as Experiment 1. After filtering, two subjects' HR data during the last von Frey pain tests were not analyzed due to uncorrectable noise.

Epinephrine and Cortisol ELISA The procedure for the blood draw and ELISA were the same as in Experiment 1.

Breathalyzer A breathalyzer test was performed to estimate BACs with BACtrack S75 Pro breathalyzer. This breathalyzer measures BAC from 0.000 to 0.400% with ± 0.005 error at 0.100% BAC (manufacture's manual). On the second study visit for alcohol administration, participants were instructed to rinse their mouth thoroughly with tap water at least twice before each breathalyzer test.

6.2 Procedures

This study consisted of three study visits and the experiment timeline for each study was depicted in Fig. 21. The interval between different QSTs was always 3 minutes. The LDI scanner was located outside the pain testing room. Therefore, participants walked a few steps after the sensors were detached. The experiment on Visit 1 and 3 took about 2.5 hours. The interval between the first and third visit was 7 ± 2 days and the second visit was one day before the third visit. The start time was 9am ± 30 min on Visit 1 and 3, and 8:30pm ± 30 min on Visit 2. This means that QST on Visit 3 occurred 12 to 12.5 hours after the last drink. Before Visits 1 and 2, participants were instructed not to drink alcohol for three days, in order to assess their baseline pain sensitivity while not under the influence of the acute alcohol and to evaluate the effect of alcohol only consumed at the laboratory, respectively. After Visit 2, the participants were again instructed not to drink

additional alcohol. Before alcohol administration, a breathalyzer test and a urine pregnancy test (female only) were conducted to ensure zero BAC and no alcohol administration to a pregnant woman, respectively.

<table>
<tr><td colspan="7">Visit 1 (Baseline) & Visit 3 (5±2 days after Visit 1): 9am-11:30am</td></tr>
<tr><td>1</td><td>2</td><td>3</td><td colspan="3">4</td><td>5</td><td>6</td><td>7</td></tr>
<tr><td rowspan="2">V/S
BAC
Blood draw</td><td rowspan="2">Survey</td><td rowspan="2"></td><td colspan="3">QST</td><td rowspan="2"></td><td rowspan="2">Survey</td><td rowspan="2">Blood draw
V/S</td></tr>
<tr><td>VF
MPT
HPT
PPT</td><td>Capsaicin
Spontaneous pain</td><td>Scan
Flare</td><td>VF
MPT</td></tr>
<tr><td></td><td>Physio</td><td colspan="3">Physio</td><td>Physio</td><td>Physio</td><td></td><td></td></tr>
<tr><td colspan="9">Visit 2 (Alcohol/Juice Administration):8:30pm ~ midnight, one day before Visit 3</td></tr>
<tr><td>1</td><td colspan="2">2</td><td colspan="3">3</td><td colspan="3">4</td></tr>
<tr><td>V/S
BAC</td><td colspan="2">Pregnancy test
(female only)</td><td colspan="3">Alcohol/Juice
(30 min)</td><td colspan="3">BAC</td></tr>
</table>

Fig. 21. Experiment 2 timeline. V/S: Vital Sign (BP, HR, BT), VF: Von Frey on the grid, MPT: Mechanical Pain Threshold, HPT: Heat Pain Threshold, PPT: Pressure Pain Threshold

A standardized alcohol administration procedure was used (57). The target BAC was 0.04% for moderate drinkers and 0.08% for binge drinkers. The amount of alcohol was calculated using the Matthews and Miller's formula: BAC = [(The number of drinks/2) × (GC/Weight in pounds)] - (0.016×the number of hours over the consumed alcohol)] (58). In their formula, GC is a gender constant, 9.0 for women and 7.5 for men, and 0.016 is a constant for alcohol metabolism rate. Vodka (80 proof) and fruit juice (18g sugar/200ml) were mixed in a 1:3 ratio.

The participants were asked to drink a third of the drink relatively quickly and to finish the rest over a 30-minute period. If the level was below the target, an additional shot was given. Once the target BAC was reached, participants stayed in the laboratory until their BAC levels were down to 0.04. Participants were only allowed to leave the laboratory with a sober friend/family member or to use a cab. All participants were compensated with money for their participation. Abstainers received $20, $20, and $25 checks for the first, second and last study visit, respectively. Moderate and binge drinkers received $20, $30, and $50 checks for the first, second and last visit, respectively. Additionally, moderate and binge drinkers received $20 in cash on the second and third visit for transportation costs. After the QST on Visit 1 and Visit 3, participants were asked to report their guess about the study purpose. Most drinkers mentioned alcohol effects whereas abstainers did not mentioned alcohol. Consequently, the study hypothesis was blinded only to the abstainers.

One male and four female experimenters conducted this experiment. One male and one female experimenter were involved in prescreening, tracking recruitment, obtaining informed consent, verifying compliance of study preparation (including breathalyzer test), and giving a reminder call for their appointment. Instruction for study preparation was the same as in the Experiment 1 except for instructions on alcohol consumption. Self-report and breathalyzer tests were used to assess their compliance of alcohol consumption. One participant whose baseline BAC was non-zero on the first study visit did not undergo the QST and the visit was rescheduled. Two female experimenters administered alcohol or juice on the second study visit. The last female experimenter who was blinded for

participants' drinking pattern and performed blood draws and the QSTs in the Experiment 1 performed all blood draw and QSTs for Experiment 2.

6.3 Statistical Analysis

A 2 (visit) by 3 (group) repeated measures MANOVA was used to examine changes in mechanical, heat, and pressure pain thresholds. Nonparametric analyses were conducted for non-normal data and any significant violation of assumption.

Sample Size Calculation

GPower 3.1.9 was used to calculate a priori sample size for MANOVA repeated measures. Large between subject effect size (f=0.5) with α of .05 Type 1 error, 80% power, and 0.6 correlation coefficient among repeated pain measures (59) were entered. The results indicated a minimum sample size was a total of 36 (n = 12 each).

Missing Data

No missing values were found in pain sensitivity, blood samples, and physiological data. All questionnaires were completed with the exception of one binge drinker who completed only half of the survey after the QST at Visit 1 and another binge drinker who did not completed the ACQ survey before the QST at Visit 3. Pairwise deletion was used.

7. RESULTS

EXPERIMENT 2

Table 4. Experiment 2 Demographics and drinking patterns

	Abstainers	Moderate drinkers	Binge Drinkers		
	M(*SD*)	*M*(*SD*)	*M*(*SD*)	*F* or *U**	*p*
N	12	12	12		
(Male/Female)	(5/7)	(6/6)	(6/6)		
Caucasian (*n*)	5	7	9		
Asian (*n*)	5	4	0		
Other (*n*)	2	1	3		
% Cigarette Current/ex-smoker	0/0	17/20	0/33		
Age	23.8(1.7)	25.3(2.3)	22.8(2.6)	3.7*	.036
Typical drinks (weekday)	-			14.5*	< .001
Men		3.8(1.2)	8.5(4.8)		
Women	-	3.2(1.7)	11.0(5.5)		
Typical drinks (weekend)	-			12.0*	< .001
Men		2.8(1.2)	7.2(3.5)		
Women	-	2.7(1.4)	6.8(1.8)		
Heaviest drinks	-			1.0*	< .001
Men		3.8(1.2)	10.5(3.3)		
Women	-	3.5(1.4)	11.3(5.0)		
Ages at first drink	-	19.8(2.7)	18.3(2.2)	45.0*	.113
Years of drink	-	4.5(3.2)	3.8(4.4)	57.0*	.379
Frequency	-	3.5(0.9)	4.0(0.9)	47.5*	.133
HSS	-	3.7(2.2)	6.9(2.6)	10.9	.003

Other ethnicity includes Latin American, African, and multi-racial. Acute drinking: alcohol consumption within 48 hours before the experiment; *:Mann-Whitney U test, HSS: Hangover Symptoms Scale.

Participants' demographic information and drinking patterns are summarized in Table 4. Each group consisted of 12 participants. The majority was Caucasian for the binge (75%) and moderate drinker groups (58.3%). Different from Experiment 1, none of the participants from the abstainer or binge drinker groups endorsed being a current smoker.

At prescreening, binge drinkers reported consuming greater amounts of alcohol during weekdays, weekends, and the heaviest drinking days compared to moderate drinkers (Table 4). The first age to drink alcohol, year of regular drinking, and frequency of alcohol use were not different between the two drinking groups. Finally, as expected, binge drinkers reported experiencing more hangover symptoms for the past 12 months.

Table 5. Experiment 2 Psychological traits

	Abstainers		Moderate drinkers		Binge drinkers	
	M	*SD*	*M*	*SD*	*M*	*SD*
DASS-DEP	5.3	4.1	4.3	6.5	5.3	6.2
DASS-ANX	4.3	4.6	4.7	5.5	2.0	2.3
DASS-STR	11.8	9.1	5.8	5.7	6.2	5.7
PSS	17.6	7.2	14.2	4.5	12.8	6.7
BIS-Total*	4.0	0.1	4.1	0.1	4.0	0.2
BIS-ATTEN*	2.7	0.2	2.7	0.3	2.7	0.3
BIS-MOTOR*	3.0	0.1	3.1	0.1	3.0	0.2
BIS-PLAN*	3.0	0.2	3.0	0.2	3.0	0.2
UPPS-Total	113.9	5.0	114.9	5.1	116.6	3.1
UPPS-Lack of Premeditation	24.8	3.3	24.3	4.4	24.5	2.7
UPSS-Urgency	31.7	3.9	33.2	3.4	33.4	2.0
UPSS-Sensation seeking	31.4^{a}	2.7	31.7^{a}	3.7	34.5^{b}	2.4
UPSS-Lack of Perseverance	26.0	3.5	25.8	2.9	24.2	2.3
ZKPQ-ImpSS	7.9	4.1	10.2	3.8	11.4	3.3

DASS (Depression Anxiety Stress Scales)-DEP: Depression, ANX: Anxiety, STR: Stress; PSS: Perceived Stress Scale; BIS (Barratt Impulsiveness Scale)-ATTEN: Inattention, MOTOR: Motor Restlessness, PLAN: Non-Planning; ACQ (Acute Craving Questionnaire-NOW)-COMP: Compulsivity; XPCT: Expectancy; PURP: Purposefulness; EMOT: Emotionality, UPPS (UPPS Impulsive Behavior Scale), ZKPQ-ImpSS: Zuckerman-Kuhlman Personality Questionnaire–Impulsive Sensation Seeking; superscripts of *a*, *b* and *c* indicate significant difference in post hoc analysis. * log transformed.

Different from Experiment 1, none of the participants from the abstainer or binge drinker groups endorsed being a current smoker. At prescreening, binge drinkers reported consuming greater amounts of alcohol during weekdays, weekends, and the heaviest drinking days compared to moderate drinkers (Table 4). The first age to drink alcohol, year of regular drinking, and frequency of alcohol use were not different between the two drinking groups. Finally, as expected, binge drinkers reported experiencing more hangover symptoms for the past 12 months.

7.1 Psychological Traits

Baseline psychological traits were compared between the groups. The Kruskal-Wallis test showed no difference in DASS-depression, anxiety, and stress scores (*p*s > .149). PSS scores were also similar between the groups, F (2, 33) = 1.90, p = .166. Except for depressive symptoms, these results are consistent with Experiment 1. In Experiment 1, binge drinkers reported more depressive symptoms than abstainers. This difference in Experiment 1 was caused by less depressed abstainers and not by more depressed binge drinkers in comparison with abstainers and binge drinkers in Experiment 2. However, the baseline psychological traits of the groups with same drinking pattern were not different between Experiment 1 and 2 (*p*s of independent *t* tests > .095). Consistent with Experiment 1, BIS total and subscale scores were not significantly different between the groups, *F*s (2, 32) < 0.54, *p*s > .591. UPPS-total and subscale scores also showed levels of impulsivity and specific dimensions of impulsivity were similar across the groups, *F*s (2, 33) < 1.40, *p*s > .262 with one exception. The binge drinkers reported engaging in more sensation seeking behaviors than the abstainer and moderate drinker groups, F (2, 33) = 3.89, p =

.030. ZKPQ-ImpSS scores reflecting both impulsivity and sensation seeking personality showed a marginal difference, F (2, 32) = 2.51, p = 0.97.

7.2 Baseline Psychological and Physiological Sates

At baseline, psychological (Kruskal-Wallis tests χ^2 < 4.05, ps > .132) and physiological states were not different between the groups, Table 6. The results of an ANOVA indicated no group difference in HR, sqrtSCL, and RR, F (2, 33), ps > .558. SCLs were similar between the groups even when adjusting for ethnicity (White = 1 vs other ethnicity = 0, p = .702). The Kruskal-Wallis test also indicated that SBP and DBP were not different between the groups (ps > .062).

Table 6. Experiment 2 Baseline physiological and psychological states

	Abstainers		Moderate drinkers		Binge drinkers	
Psychological State	*M*	*SD*	*M*	*SD*	*M*	*SD*
STAI	9.3	3.2	8.4	1.6	7.5	1.9
PANAS-PA	30.4	5.4	30.3	6.3	31.0	9.6
PANAS-NA	12.9	2.9	11.4	2.5	11.8	2.8
SAM-V	5.4	1.4	5.8	1.7	6.3	1.3
SAM-A	2.6	2.0	3.6	1.9	3.0	2.2
SAM-D	6.0	1.8	7.2	1.6	7.3	1.2
Physiological State						
HR(beat/min)	72.0	10.1	71.7	10.8	73.4	8.5
sqrtSCL(μS)	2.3	0.5	2.3	0.9	2.6	0.8
RR(breath/min)	13.0	4.1	13.3	2.9	12.6	1.9
SBP(mmHg)*	113.1	13.5	110.8	10.8	109.3	12.7
DBP(mmHg)*	72.4	8.1	73.8	9.4	70.5	10.2

STAI: State-Trait Anxiety Inventory-6, PANAS (Positive and Negative Affect Schedule)-PA: Positive affect, NA- Negative affect, SAM (Self-Assessment Manikin)-V: Valence, A-Arousal, D: Dominance; HR: Heart Rate, SCL: Skin Conductance Level, RR: Respiration Rate; SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure;*: the Kruskal-Wallis test followed by the Mann-Whitney *U*.

7.3 Influence of Alcohol on Visit 3

On Visit 3, all participants' BACs were zero prior to pain testing. Alcohol's stimulant and sedative effects were measured with the BAES and acute hangover symptoms were measured with the ACQ. About 12 hours after alcohol consumption (moderate drinkers to 0.04% and binge drinkers to 0.08%), reported stimulant and sedative effects of alcohol were similar between the moderate and the binge drinker groups (Mann-Whitney U = 57 for the stimulant effect and 72 for the sedative effect, ps > .374). The means of the BAES-stimulant effect scores were 14.0 (SD = 20.0) for the moderate drinker group and 19.8 (SD = 21.2) for the binge drinker group. The means of the BAES-sedative effect scores were 10.8 (SD = 13.4) for the moderate drinker group and 11.6 (SD = 14.2) for the binge drinker group. Different from Experiment 1, AHS scores were not different between the moderate (M = 5.8, SD = 2.9) and binge drinker groups (M = 4.9, SD = 5.3). Mann-Whitney tests indicated that AHS scores were higher in binge drinkers with acute alcohol use in Experiment 1 than binge drinkers with acute alcohol use in Experiment 2. During the exit interview, 75% of binge drinkers reported the amount of alcohol consumed in the laboratory was less than their typical drinking and 25% reported about the same. Subsequently, all binge drinkers reported less hangover symptoms on Visit 3 than their typical morning after drinking.

7.4 Pain Sensitivity

First, peripheral pain sensitization was evaluated. A 2 (visit) by 3 (group) repeated measures MANOVA was conducted for mechanical (log transformed), heat, and pressure pain thresholds (log transformed), separately (Fig. 22). The results indicated no main

effect of visit (*p*s > .140), group (*p*s > .295), or interaction (*p*s > .241) with one exception. Time was significant for pressure pain threshold (Wilks' Λ = .883, p = .044), suggesting that pressure pain was reduced by 14% at Visit 3. Although not significant, the moderate drinkers showed the most reduction (27%), followed by binge drinkers (15%) and abstainers (5%). In entering gender as a factor or covariate, the result remained unchanged. In comparison with Experiment 1 results, the results of mechanical and heat pain thresholds consistently showed no association with alcohol withdrawal, but failed to replicate the enhancement of pressure pain thresholds during alcohol withdrawal.

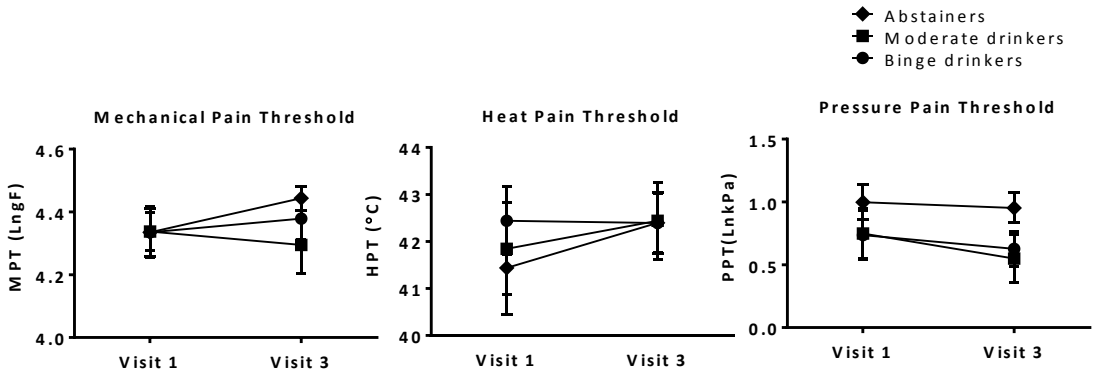

Fig. 22. Changes in mechanical, heat, and pressure pain threshold from Visit 1 to Visit 3. Error bars = SEM.

Capsaicin-induced Spontaneous Pain

Second, spontaneous pain intensity and unpleasantness were compared between the groups on Visit 1 and Visit 3. Because the data distributions were not normal and uncorrectable with transformation, the average and peak pain intensity were computed for

each visit and nonparametric analyses were conducted. The pain intensity and unpleasantness ratings reported every two minute are depicted in Fig. 23.

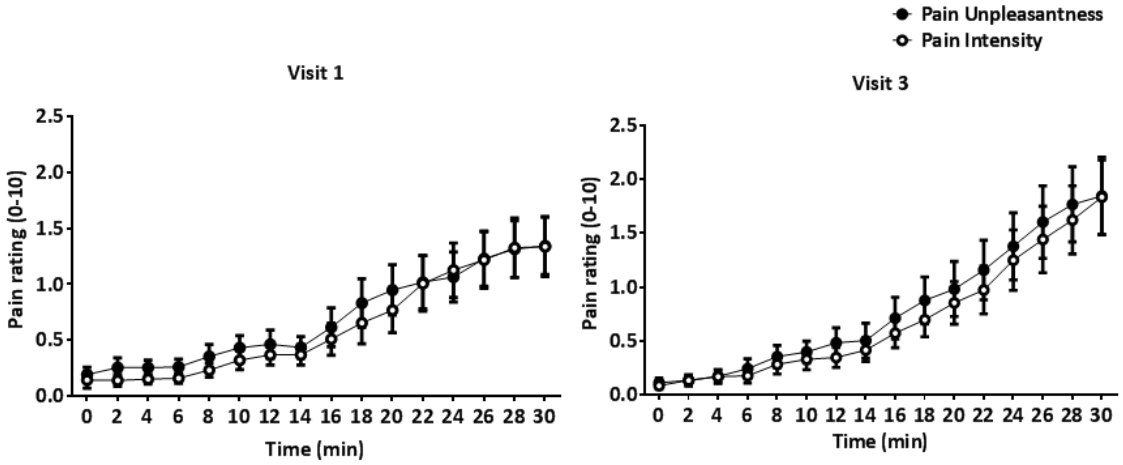

Fig. 23. Average pain intensity and unpleasantness ratings every two minute on Visit 1 and Visit 3. Error bars = SEM.

Kruskal-Wallis tests were conducted to compare average capsaicin-induced pain on Visit 1 and changes on Visit 3 (Fig. 24). Neither pain intensity (p = .649) nor unpleasantness (p = .700) was different between the groups on Visit 1. There were also no group differences in changes in pain intensity (p = .669) or unpleasantness (p = .563) on Visit 3. However, changes in average pain intensity showed a trend toward increasing in binge drinkers.

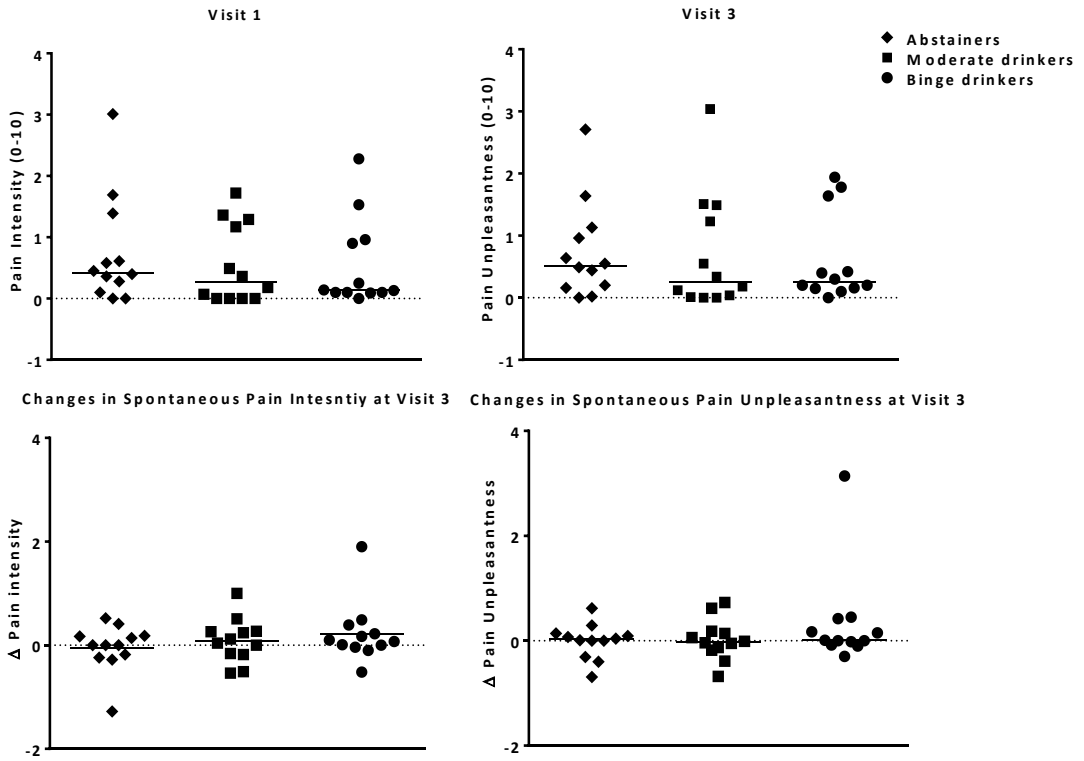

Fig. 24. Scatter plots for each individual's average capsaicin-induced pain intensity (top left) and unpleasantness (top right) on Visit 1 and change in pain intensity (bottom left) and unpleasantness (bottom right) on Visit 3. Mid-lines represent medians.

Peak pain intensity and unpleasantness during capsaicin application were compared between the groups (Fig. 25). The results of Kruskal-Wallis tests indicated that neither peak pain intensity (p = .762) nor unpleasantness (p = .805) was different between the groups on Visit 1. Changes in pain intensity (p = .296) and unpleasantness (p = .732) on Visit 3 were also not different between the groups.

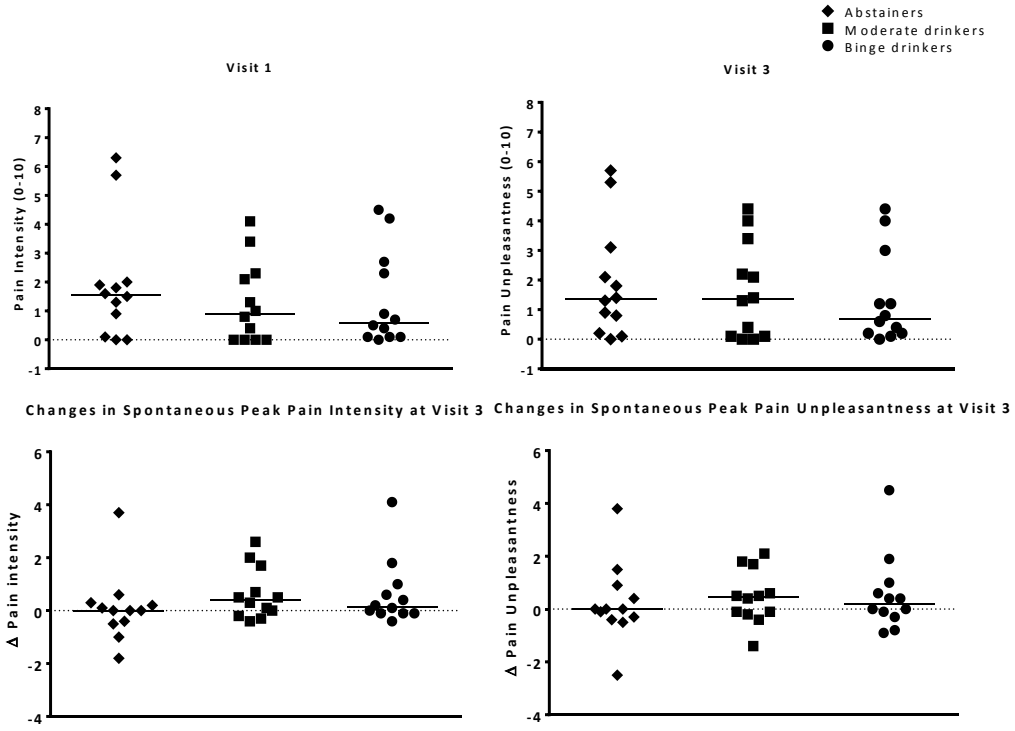

Fig. 25. Scatter plots for each individual's peak pain intensity (top left) and unpleasantness (top right) during capsaicin application on Visit 1. Scatter plots for change in pain intensity (bottom left) and unpleasantness (bottom right) on Visit 3. Mid-lines represent medians

Exploratory pattern analyses were conducted to examine whether the average number of drinks during the weekends, weekdays, and the heaviest drinking days for the past month predicted capsaicin-induced pain intensity and unpleasantness. The exploratory analyses found a significant result (Fig. 26). Up to a half-standard drink predicted a slight decrease in peak pain intensity during capsaicin application, but thereafter, more drinks predicted an increased in peak pain intensity.

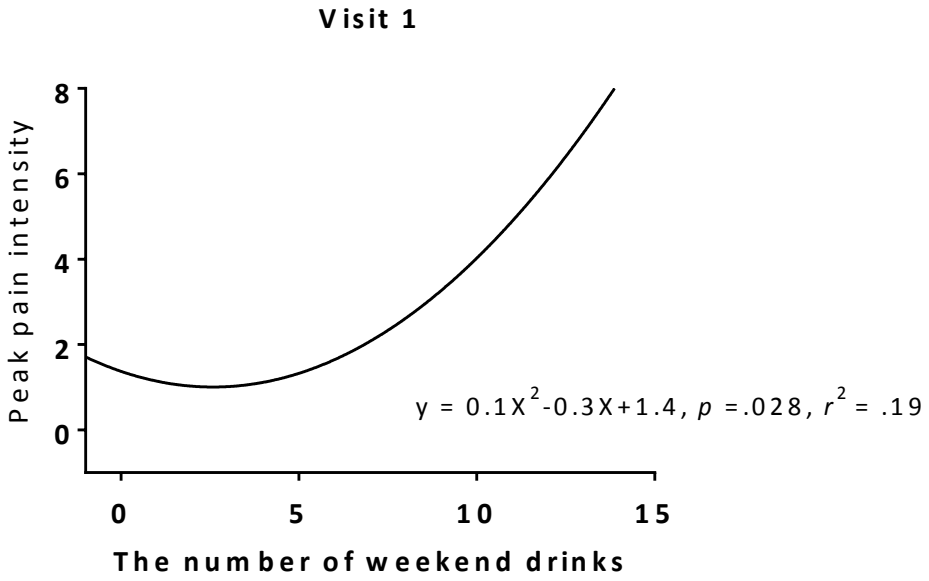

Fig. 26. Predicting capsaicin induced-peak pain intensity from the number of drinks during weekends

Capsaicin-induced Flare

On Visit 1, sizes of capsaicin-induced flare were not different between the groups (Kruskal-Wallis $\chi^2 = 2.36$, $p = .308$). Changes in flare size on Visit 3 were marginally different between the three groups (Kruskal-Wallis $\chi^2 = 4.98$, $p = .083$). Post-hoc Mann-Whitney *U* tests indicated a significant difference between the moderate and binge drinker groups, ($U = 36.0$, $p = .039$), Fig. 27. On Visit 3, the flare size was increased in the binge drinker group ($M = 1.3\text{cm}^2$, $SD = 2.5$) whereas the flare was decreased in the abstainers ($M = -0.5\text{cm}^2$, $SD = 2.1$) and moderate drinkers groups ($M = -1.4\text{cm}^2$, $SD = 4.0$). When comparing intensity of the flare, the results consistently showed no baseline difference ($p = .566$). However, changes in intensity on Visit 3 were marginally different between the groups ($p = .089$). Post-hoc Mann-Whitney *U* tests indicated a significant difference

between the moderate and binge drinker groups (U = 37.0, p = .045). On Visit 3, the flare intensity was increased in the binge drinker group (M = 332.3PU, SD = 486.3) whereas the flare was decreased in the moderate drinker group (M = -42.3PU, SD = 288.8). Abstainers showed a slight increase in flare intensity on Visit 3 (M = 10.0PU, SD = 297.4).

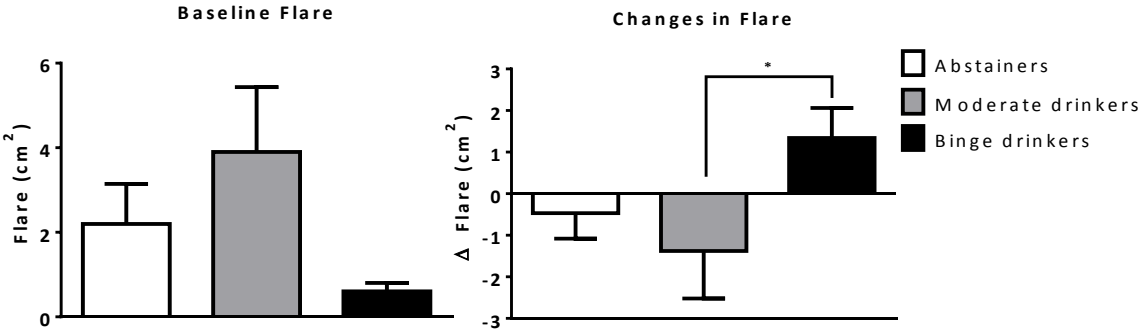

Fig. 27. Comparison of flare size on Visit 1 and changes in flare size on Visit 3. Error bars = SEM.

Area of Secondary Allodynia

Secondary allodynia was compared between the groups. Before comparing secondary allodynia, baseline pain responses to mechanical stimuli (26gF) on the furthest (referencing) point were evaluated. The results of Kruskal-Wallis tests indicated no group differences on dominant and non-dominant feet (ps >.633). Participants reported the mechanical stimulation on the non-dominant foot was overall not painful on Visit 1 (M = 0.5cm, SD = 0.5) and Visit 3 (M = 0.3cm, SD = 0.4). This was also true for the dominant foot on Visit 1 (M = 0.5cm, SD = 0.5) and Visit 3 (M = 0.4cm, SD = 0.4).

Areas of secondary allodynia were calculated in two ways. First, the areas were calculated with boundaries of any persistent increase in pain intensity from the furthest point on each spoke. On Visit 1, no significant difference in the area of secondary allodynia was found, Kruskal-Wallis $\chi^2 = 2.45$, $p = .294$. The means for the areas were 32.6cm^2 ($SD = 22.5$) for the abstainer group, 42.3cm^2 ($SD = 32.4$) for the moderate drinker group, and 27.2cm^2 ($SD = 29.6$) for the binge drinker group. An ANOVA was conducted to compare change scores of the area from D1 and D3 (normal distribution, Shapiro-Wilk = .98, $p = .641$). The result indicated no significant group difference, F (2, 33) = 0.58, p = .568, Fig. 28. On average, the areas remained unchanged ($M = 0.5$, $SD = 23.2$).

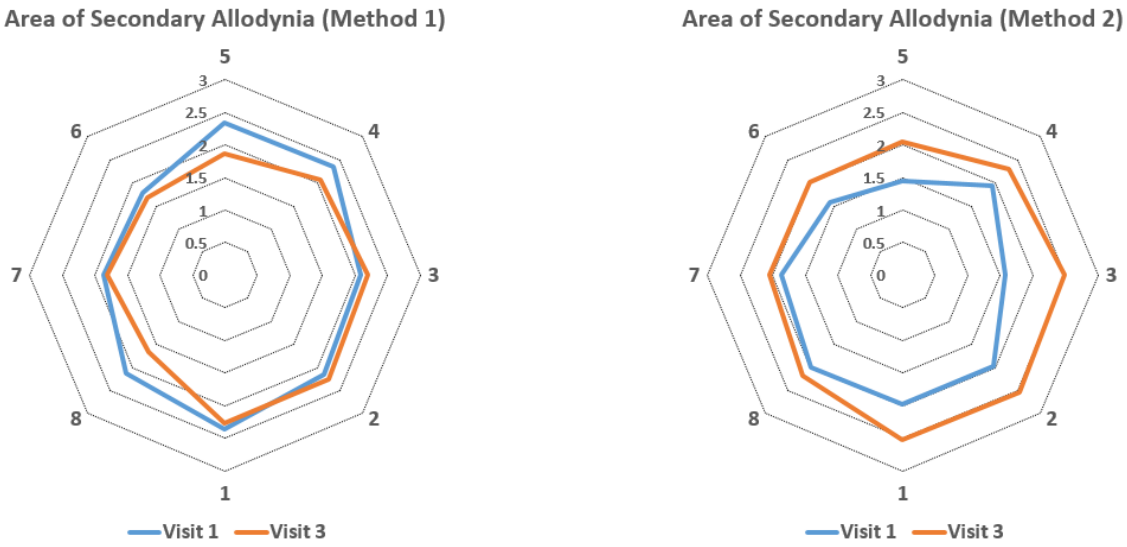

Fig. 28. Graphical depiction of the areas of secondary allodynia on the capsaicin application site using two methods for Visit 1 and Visit 3

Second, the areas were calculated with boundaries of any persistent increase in pain intensity from the baseline. Consistent with the results with the first method, the areas

on Visit 1 were not different between the groups, Kruskal-Wallis $\chi^2 = 0.48$, $p = .787$. The means for the areas were 23.0cm^2 (*SD* = 18.0) for the abstainer group, 30.7cm^2 (*SD* = 27.7) for the moderate drinker group, and 24.9cm^2 (*SD* = 27.5) for the binge drinker group. An ANOVA was conducted to compare the change scores of the area from D1 and D3 (Shapiro-Wilk = .95, $p = .072$). The results indicated no significant group difference, F (2, 33) = 0.50, $p = .610$, Fig. 29. However, when using this method, the mean of the areas increased to 8.3cm^2 (*SD* = 19.8) on Visit 3.

The second method assumes that pain sensitivity would not be the same across the foot surface. The results of the repeated measures MANOVA showed that pain ratings on the spoke 3 on Visit 1, $\Lambda = 0.56$, F (9, 27) = 2.40, $p = .038$, and the spoke 4 on Visit 3, $\Lambda = 0.54$, F (9, 27) = 2.59, $p = .027$, were significantly increased when it got closer to the center. Yet, pain ratings on the other spokes showed no significant changes (ps > .062), Fig. 29.

Areas of secondary allodynia were also calculated on the non-capsaicin application site. The results were similar to the capsaicin application foot, Kruskal-Wallis $\chi^2 = 1.65$, $p = .438$. Notably, the areas of allodynia on non-capsaicin application sites were not zero on Visit 1, suggesting the occurrence of secondary allodynia on the contralateral side. The means for the areas were 19.5cm^2 (*SD* = 19.5) for the abstainer group, 32.8cm^2 (*SD* = 34.7) for the moderate drinker group, and 20.6cm^2 (*SD* = 31.2) for the binge drinker group. Changes in the area from D1 and D3 were compared between the groups and the results found no significant group difference (Kruskal-Wallis = 0.18, $p = .915$). On average, the areas remained unchanged or decreased slightly (*M* = -1.8, *SD* = 9.0) on Visit 3.

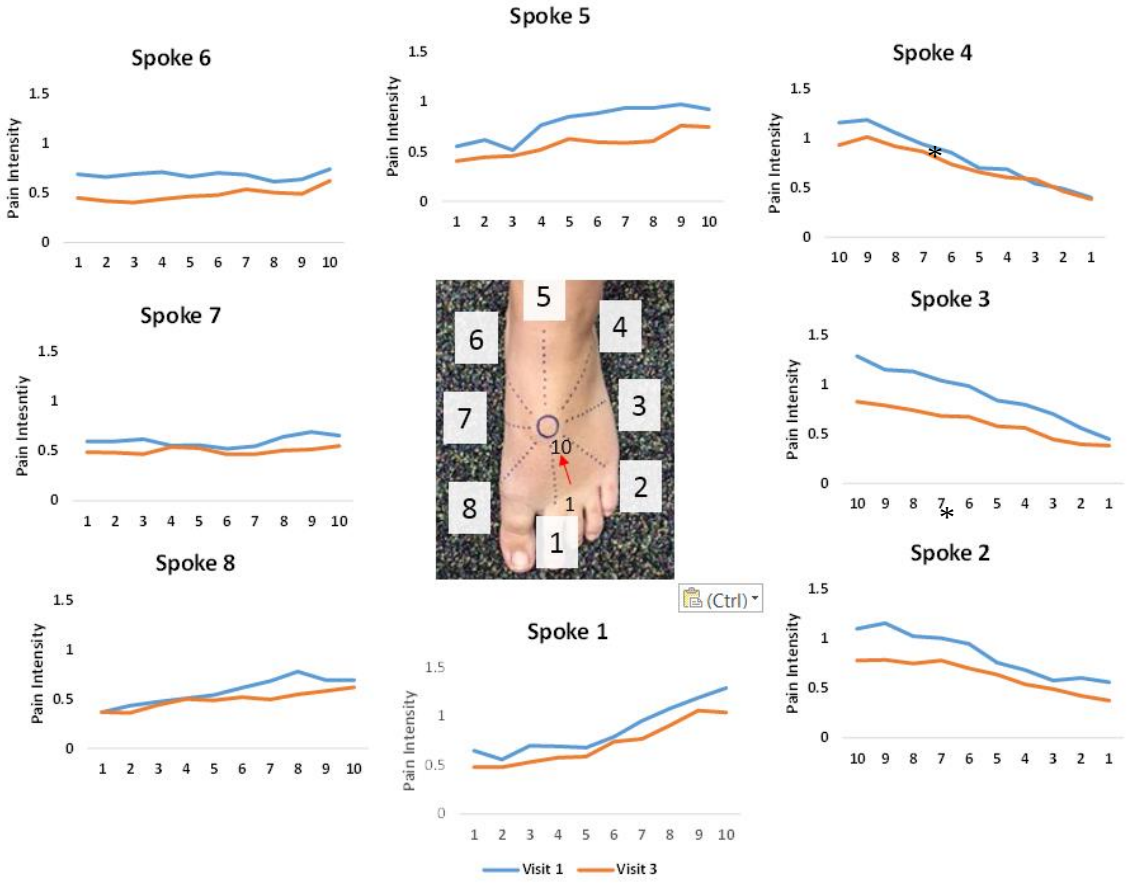

Fig. 29. Pain intensity ratings to 26g von Frey on non-dominant foot before capsaicin application

In examining areas on the unaffected site with the second method, the areas on Visit 1 were not different between the groups, Kruskal-Wallis $\chi^2 = 0.80$, $p = .670$. The means for the areas were 9.7cm^2 (*SD* = 6.9) for the abstainer group, 18.0cm^2 (*SD* = 20.7) for the moderate drinker group, and 15.2cm^2 (*SD* = 24.5) for the binge drinker group. An ANOVA was conducted to compare the change scores of the area from D1 and D3 (Shapiro-Wilk = .95, $p = .072$). The results indicated no significant group difference (p =

.831). Using this method, the mean of the areas decreased to 4.2cm^2 (*SD* = 18.2) on Visit 3.

Primary Allodynia

Changes in mechanical pain thresholds on the capsaicin application sites and the vehicle sites were compared between the groups. The results of an ANOVA indicated that changes in mechanical pain thresholds on Visit 1 and Visit 3 did not differ between the groups (*p*s > .093). On average, mechanical pain thresholds were reduced by 19% on the capsaicin application site on both Visit 1 and Visit 3. In contrast, the thresholds were increased by 4% and 1% on the vehicle sites on Visit 1 and Visit 3, respectively.

7.5 The Role of Stress Hormones in Pain Sensitivity

For the group comparison with epinephrine levels, the results of a 2 (visit) by 2 (pre and post pain testing within visits) by 3 (group) repeated measures MANOVA indicated no significant main effect of visits ($p = .650$) and pain testing ($p = .325$) and no significant interactions (*p*s > .503), Fig. 30. The non-significant group by visit interaction indicated that alcohol withdrawal did not increase epinephrine levels. To note, the visit by pain testing interaction was marginally significant, $\Lambda = .603$, $F\ (1, 33) = 3.56$, $p = .068$. Pain testing increased the average epinephrine levels from 8.6 (*SD* = 3.0) to 9.4 (*SD* = 2.9) on Visit 1 whereas pain testing decreased the levels from 9.2 (*SD* = 2.7) to 9.1 (*SD* = 2.2) on Visit 3. There was a main effect of group, $F\ (2, 33) = 5.24$, $p = .011$, partial $\eta^2 = .241$. LSD post hoc tests indicated that the epinephrine levels were lower in the moderate drinker group than the binge drinker ($p = .006$) and abstainer groups ($p = .012$). The levels were

not different between the binge drinker and abstainer groups (p = .806), Fig. 30, bottom. The findings of elevated overall epinephrine levels in the binge drinker group compared to the abstainer or moderate drinker groups are consistent with Experiment 1. However, the elevated basal epinephrine did not alter pain sensitivity in binge drinkers at baseline or during alcohol withdrawal because pain sensitivity was not different at baseline and did not change pain sensitivity during alcohol withdrawal.

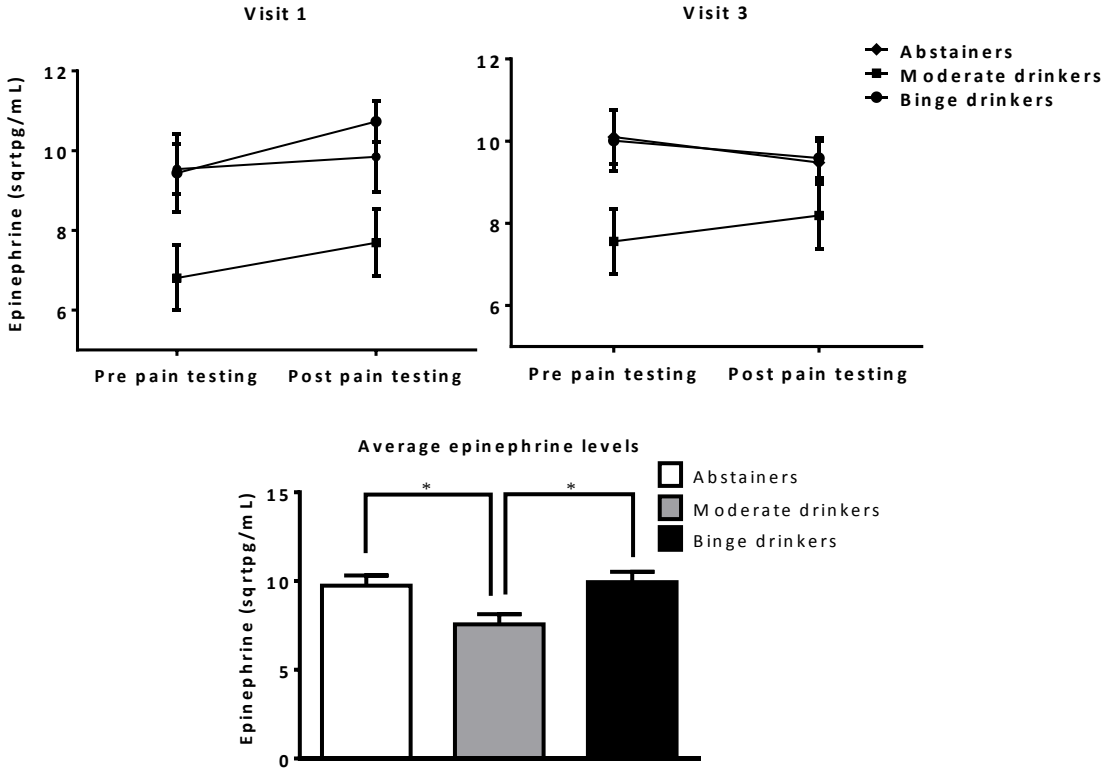

Fig. 30. Changes in epinephrine levels before and after pain testing on Visit 1 (top left) and Visit 3 (top right), and comparison of overall epinephrine levels between the groups (bottom), Error bars = SEM.

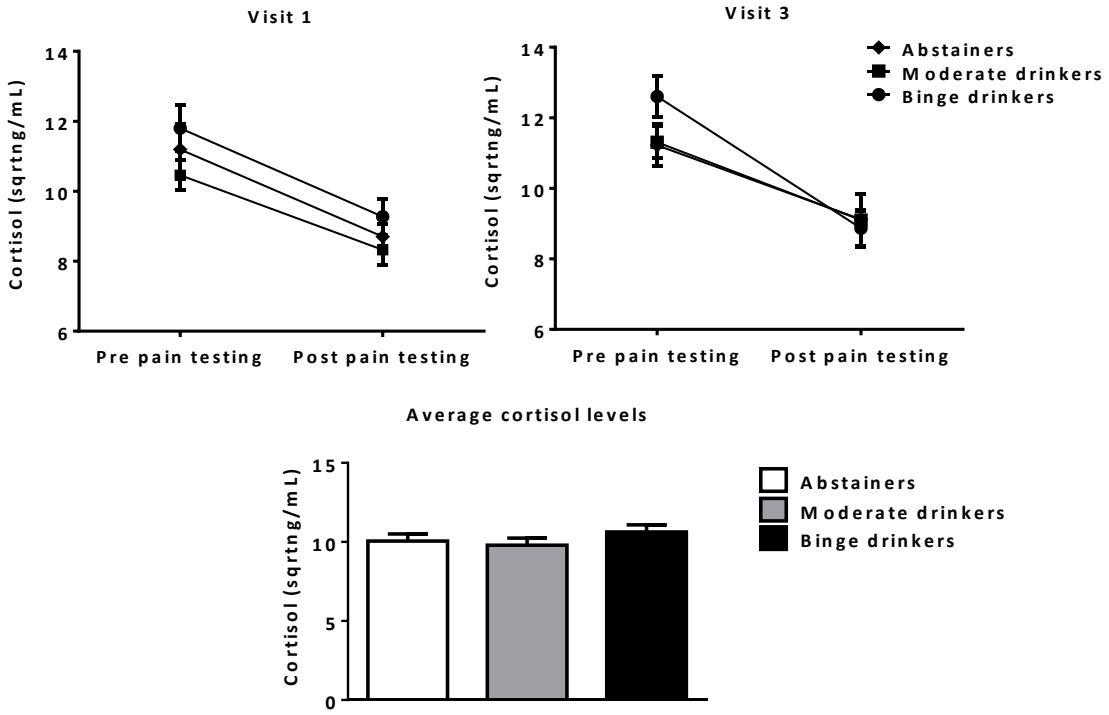

Fig. 31. Changes in cortisol levels before and after pain testing on Visit 1 (top left) and Visit 3 (top right), and comparison of overall cortisol levels between the groups (bottom), Error bars = SEM.

For the group comparison with cortisol levels, the results of a 2 (visit) by 2 (pre and post pain testing within visits) by 3 (group) repeated measures MANOVA indicated a main effect of visit, $\Lambda = .822$, F (1, 33) = 7.13, $p = .012$, and pain testing, $\Lambda = .233$, F (1, 33) = 108.47, $p < .001$, Fig. 31. Overall, cortisol levels increased from 10.0 (*SD* =1.6) on Visit 1 to 10.4 (*SD* =1.6) on Visit 3. However, there was no significant group by visit interaction ($p = .200$), suggesting that alcohol withdrawal did not increase cortisol levels. Additionally, pain testing did not increase the cortisol levels. The average cortisol levels decreased from 11.4 (*SD* = 1.9) to 8.9 (*SD* = 1.6). All the other results were not

significant (*p*s > .177). These findings of no significant group difference and decreases in cortisol after pain testing are consistent with Experiment 1.

7.6 The Role of Negative Affect in Pain Sensitivity

The results of Kruskal-Wallis tests indicated that changes in anxiety after pain testing were not significantly different between the groups on Visit 1 ($\chi^2 = 0.10$, $p = .950$) and Visit 3 ($\chi^2 = 0.80$, $p = .671$). Anxiety levels on average increased after pain testing on Visit 1 ($M = 1.1$, $SD = 2.1$) and decreased on Visit 3 ($M = -0.4$, $SD = 3.7$). Changes in baseline anxiety levels from Visit 1 to Visit 3 were not different between the groups, $\chi^2 = 1.56$, $p = .458$. This suggests that anxiety levels were not higher in the morning after binge drinking compared to the morning after no alcohol or moderate amount of alcohol consumption.

Spearman rho correlations were computed to examine whether the binge drinkers' state anxiety at baseline were related to all pain sensitivity indices at Visit 1. The results indicated no significant relationships (*p*s > .170) except a marginal relationship between state anxiety and capsaicin-induced pain unpleasantness in the abstainer group ($r = .50$, $p = .064$). On Visit 1, changes in anxiety levels and pain sensitivity were also unrelated in all groups (*p*s > .266). However, higher levels of capsaicin-induced pain intensity ($r = .61$, $p = .037$) and unpleasantness ($r = .61$, $p = .036$) were associated with greater increases in anxiety in the moderate drinker group on Visit 3.

Next, Spearman rho correlations were computed to examine whether changes in baseline state anxiety on Visit 3 from Visit 1 would be related to pain sensitivity. The results indicated more induced anxiety was associated with increased heat pain thresholds

in the binge drinker group on Visit 3 (r = .62, p = .033), suggesting that alcohol withdrawal-induced anxiety would have a hypoalgesic effect. Additionally, more induced baseline anxiety on Visit 3 showed a trend towards less capsaicin induced pain intensity (r = -.53, p = .065), and unpleasantness in the abstainer groups (r = -.56, p = .076). No other relationships were significant across the other groups (ps > .105).

In sum, binge drinking did not increase the level of state anxiety the morning after drinking. Unexpectedly, higher levels of withdrawal induced anxiety were associated with reduced heat pain sensitivity in the binge drinker group and greater capsaicin-induced spontaneous pain was associated with more induction of anxiety the morning after drinking moderate amounts of alcohol.

7.7 Psychological Responses to Pain Testing

First, changes in SAM-valence scores were compared between the groups on Visit 1 (Fig. 32, top left) and Visit 3 (Fig. 32, top right). The result of a 5 (time) by 3 (group) repeated measures ANOVA showed a significant change over time, F (4, 109) = 5.20, p = .002, Greenhouse-Geisser ε = .66, without significant interaction (p = .430). A linear function best fit the data, suggesting that unpleasant emotion decreased over time, F (1, 33) = 12.22, p = .001. Additionally, the main effect of group was significant, F (1, 33) = 5.60, p = .008. The results of post hoc LSD indicated that the moderate (M = 6.8, SD = 1.3) and binge drinker groups (M = 6.8, SD = 1.3) showed higher levels of unpleasantness than the abstainer group (M = 5.2, SD = 1.3, ps < .007).

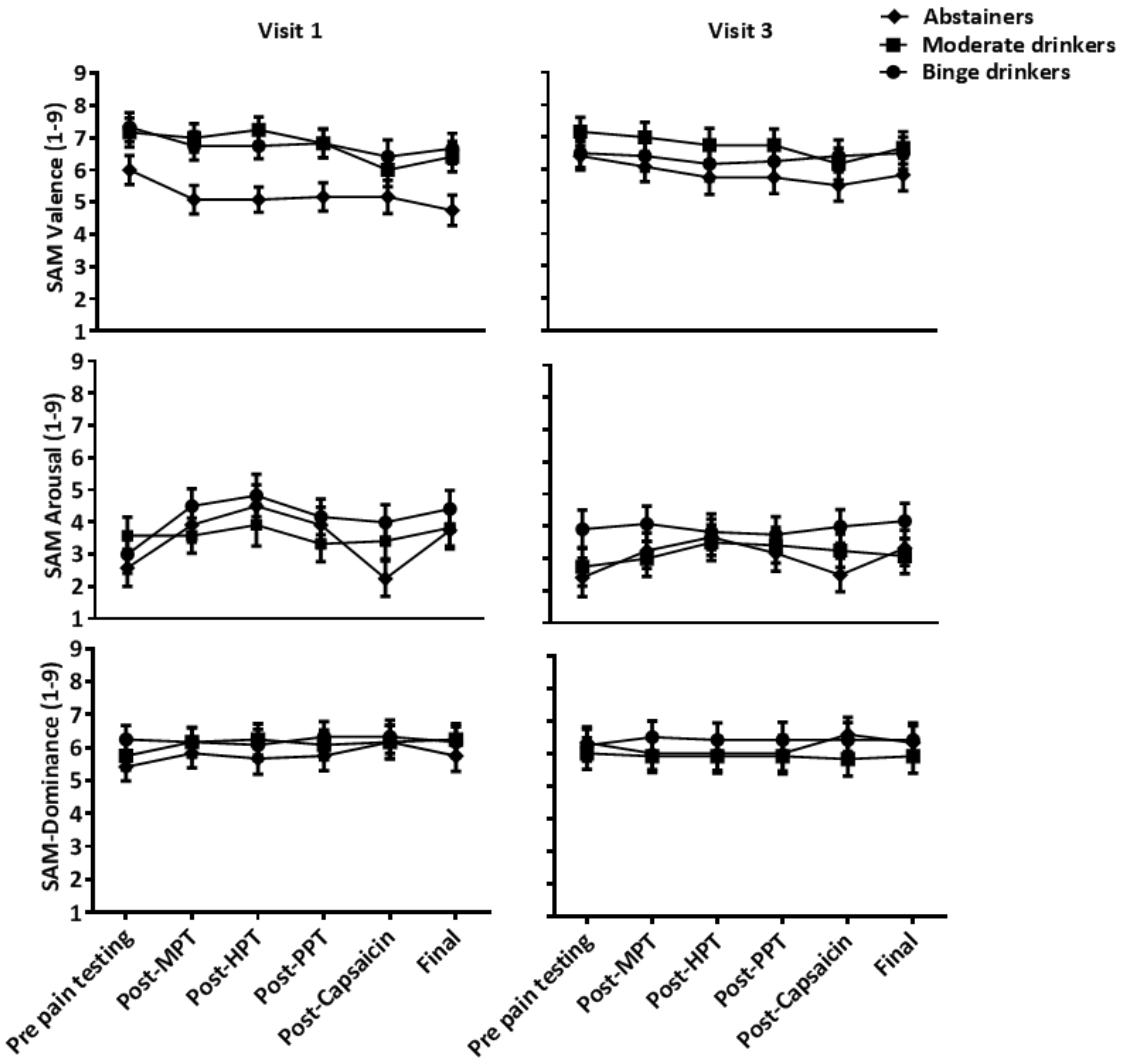

Fig. 32. Changes in the SAM-valence, arousal, and dominance in response to pain tests on Visit 1 (left) and Visit 3 (right), Error bars = SEM.

In conducting 5 (time) by 3 (group) repeated measures ANOVAs for all the others, time was a significant main effect for the SAM-arousal on Visit 1 ($p < .005$), but not for the SAM-arousal on Visit 3 ($p = .218$) and SAM-dominance on Visit 1 ($p = .127$) and Visit 3 ($p = .841$). A cubic function best fit the data, $F(1, 33) = 17.01$, $p < .001$, suggesting that levels of perceived arousal were increased up to heat pain threshold testing, decreased

after pressure pain thresholds and capsaicin application, and then slightly increased after the last mechanical pain threshold test (Fig. 32, middle left). In contrast, perceived arousal did not change over repeated pain tests on Visit 3 (Fig. 32, middle right). Consistent with Experiment 1, perceived dominance remained unchanged over time on Visit 1 (Fig. 32, bottom left) and Visit 3 (Fig. 32, bottom right).

7.8 Physiological Responses to Pain Testing

For HRs, the results of a 2 (visit) by 2 (pre and post pain testing within visits) by 3 (group) repeated measures MANOVA indicated a significant main effect of pain testing, $\Lambda = .41$, F (2, 31) = 22.77, $p < .001$, Fig. 33, top. A linear function best fit the data, suggesting overall decrease in HR over time, F (1, 32) = 24.4, $p < .001$. There were no other significant main or interaction effects (ps > .052), suggesting no alcohol withdrawal induced changes in HR.

The results of a 2 (visit) by 2 (pre and post pain testing within visits) by 3 (group) repeated measures MANOVA with SCLs indicated a significant main effect of pain testing, $\Lambda = .16$, F (2, 32) = 83.17, $p < .001$, Fig. 33, middle. A quadratic function best fit the data, suggesting an increase in SCL during pain testing and then a decrease afterwards, F (1, 32) = 169.6, $p < .001$. There were no other significant main or interaction effects, ps > .082, suggesting no alcohol withdrawal-induced changes in SCL. Additionally, the results of a repeated measures MANOVA showed no difference for SCRs to mechanical, heat, and pressure pain thresholds (ps >.190).

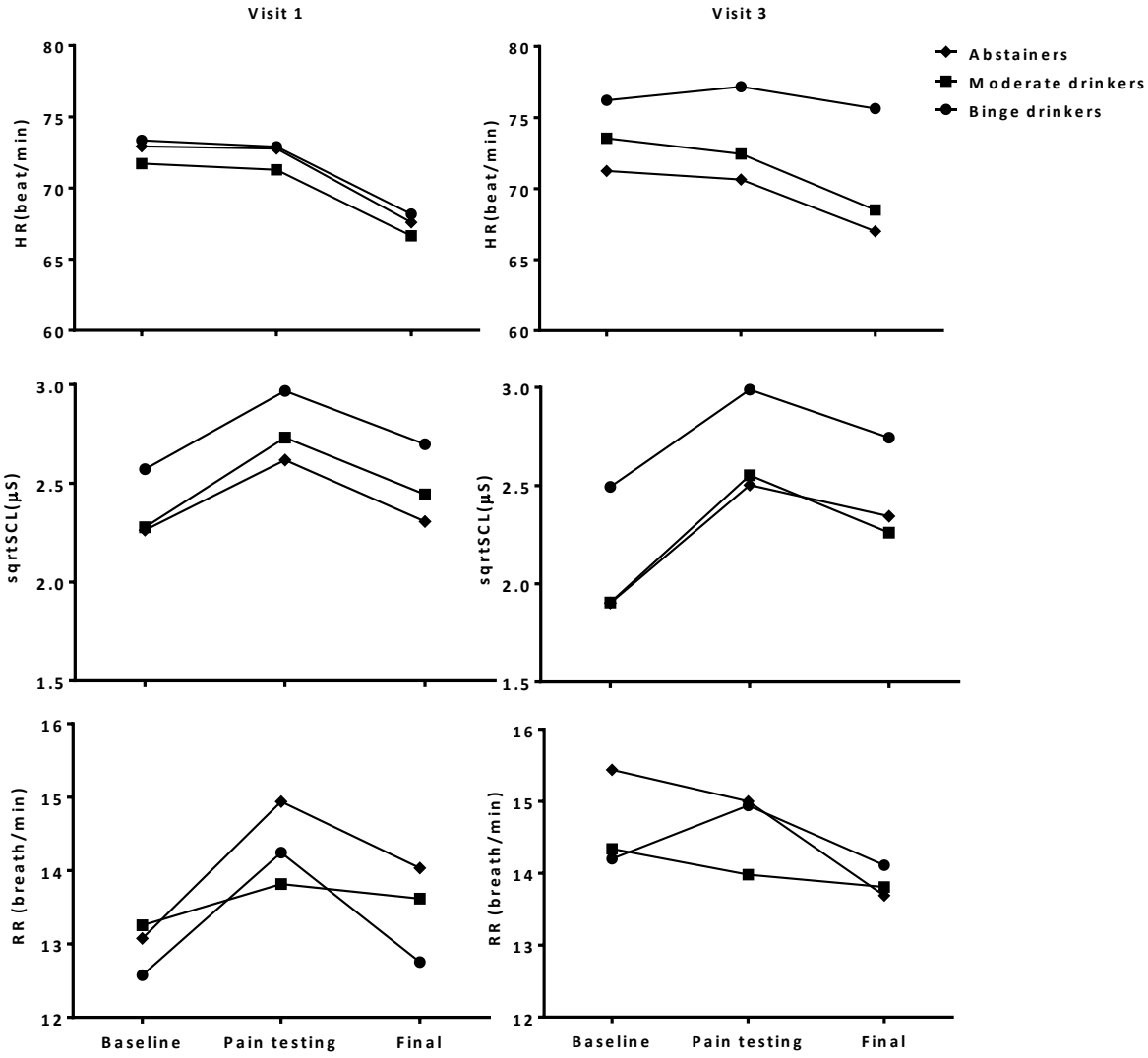

Fig. 33. Changes in HR, SCL, and RR before, during, and after pain testing on Visit 1 and Visit 3

The results of a 2 (visit) by 2 (pre and post pain testing within visits) by 3 (group) repeated measures MANOVA with RRs indicated significant main effects of visit, Λ = .65, F (1, 32) = 17.1, $p < .001$ and pain testing, Λ = .71, F (2, 31) = 6.31, p = .005. In addition, a visit by pain testing interaction was significant, Λ = .81, F (2, 31) = 3.75, p

=.035. On Visit 1, RRs were elevated during pain testing (M = 14.3, SD = 1.8), then decreased afterwards (M = 13.4, SD = 2.5) compared to baseline (M = 13.0, SD = 3.1). On Visit 3, RR were elevated before (M = 14.7, SD = 2.6) and during pain testing (M = 14.6, SD = 2.2), then decreased (M = 13.9, SD = 2.9). No other significant main or interaction effects were found, ps > .272, suggesting no alcohol withdrawal induced changes in RR.

Two 2 (visit) by 2 (pre and post pain testing within visits) by 3 (group) repeated MANOVA were conducted for SBP and DBP. The results indicated no significant main or interaction effects for SBP, ps > .054, but a main effect of pain testing for DBP, Λ = .62, F (1, 33) = 20.0, p < .001. DBP was on average increased from 71.9mmHg to 75.9mmHg after pain testing. The non-significant interactions with group suggested alcohol withdrawal did not influence SBP and DBP.

7.9 The Role of Alcohol Withdrawal and Pain in Alcohol Craving

Kruskal-Wallis tests were conducted to compare baseline craving on Visit 1 (Fig. 34). The total ACQ scores were significantly different between the groups, χ^2 = 8.9, p = .012. Consistent with Experiment 1's findings, the results of post-hoc Mann-Whitney tests showed that the moderate (U = 32.0, p = .020) and binge drinkers (U = 25.5, p = .006) reported greater alcohol craving than the abstainers. Alcohol craving was not different between moderate and binge drinkers (U = 60.0, p = .514). The baseline ACQ subscale scores were also compared. The results of Kruskal-Wallis tests indicated group difference in ACQ-XPCT scores (χ^2 = 10.0, p = .007) and marginal difference in ACQ-PURP (χ^2 = 6.0, p = .051), but no differences for the others (ps > .085). The results of post-hoc Mann-Whitney tests indicated that the moderate (p = .033) and binge drinkers (p = .003) reported

having greater positive expectancy about alcohol than abstainers, but only binge drinkers reported having stronger urges to drink with actual intent and planning to drink (p = .024).

Changes in the total and subscale ACQ scores from Visit 1 to Visit 3 were compared between the groups (Fig. 34). The results of Kruskal-Wallis tests showed no significant group difference (ps >.100). Consequently, alcohol withdrawal did not enhance craving in young adult binge drinkers. When comparing changes in the total and subscale ACQ scores before and after pain testing, no significant group difference was found on Visit 1 (ps > .186) or Visit 3 (ps > .404). In sum, pain did not increase alcohol craving in young adult binge drinkers regardless of the acute alcohol use episode.

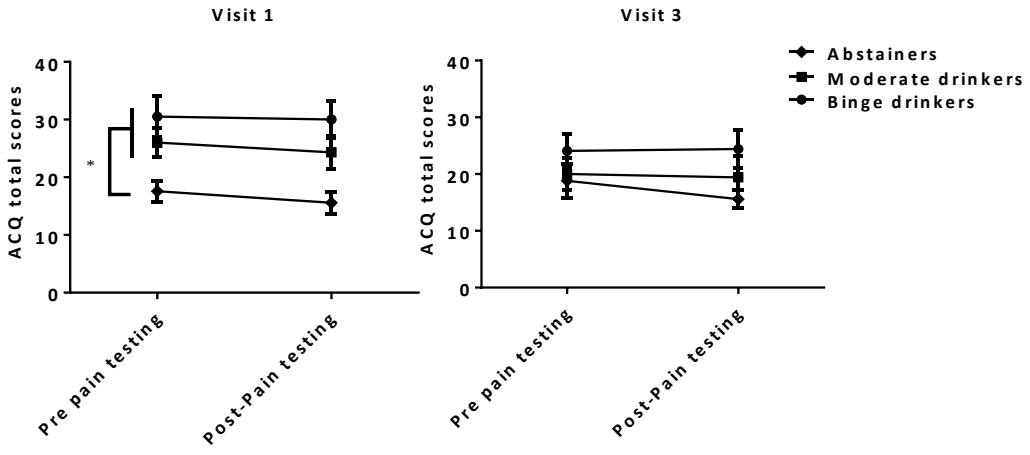

Fig. 34. Changes in ACQ scores before and after pain testing on Visit 1 and Visit 3

8. DISCUSSION

This experiment had four objectives. The first objective was to determine whether alcohol withdrawal-induced hyperalgesia would occur in young adult binge drinkers. The second objective was to examine whether stress hormones (i.e., epinephrine and cortisol) would be associated with alcohol withdrawal induced hyperalgesia. Third, the current study examined whether negative affect during alcohol withdrawal would enhance withdrawal-induced hyperalgesia. The last objective was to examine whether alcohol withdrawal and pain would enhance alcohol craving. It was hypothesized that binge drinkers would show pain hypersensitivity during acute alcohol withdrawal, plasma epinephrine and cortisol would be increased in binge drinkers and their levels would be further elevated during alcohol withdrawal, higher levels of negative affect during alcohol withdrawal would enhance alcohol withdrawal-induced hyperalgesia, and lastly, pain during alcohol withdrawal would increase craving.

Experiment 1 was conducted to test these hypotheses with a cross-sectional design focusing on peripheral pain sensitivity. Experiment 2 was a within-subject design testing both peripheral and central pain sensitivity. Experiment 1 showed that sensitivity to pressure pain was associated with binge drinking. Binge drinkers showed a slight decrease in pressure pain threshold and moderate drinkers showed a slight increase in pressure pain threshold. After drinking, binge drinkers' pressure pain thresholds were further decreased. Sensitivity to cutaneous mechanical and heat pain did not altered by binge drinking. Experiment 2 consistently showed that binge drinkers' cutaneous mechanical and heat pain thresholds were similar to abstainers' and moderate drinkers', but it failed to replicate

pressure pain threshold results. Capsaicin induced spontaneous pain intensity and unpleasantness were not different between the groups. However, exploratory pattern analysis showed a J-shape relationship between peak pain intensity and the number of drinks on the weekend. Additionally, the size and intensity of capsaicin-induced flare were increased on the morning after binge drinking. Lastly, areas of secondary allodynia were not different between the groups. Therefore, binge drinking and acute alcohol withdrawal did not alter central pain sensitivity. For the second hypothesis, Experiment 1 and 2 both showed elevated epinephrine levels in the binge drinkers compared to the moderate drinkers, but the levels were not further elevated by acute alcohol withdrawal after binge drinking. Both experiments also consistently showed that cortisol levels were unrelated to binge drinking. Different from the third hypothesis, Experiment 1 found that greater state anxiety at baseline was associated with lower pressure pain thresholds only in abstainers. Experiment 2 unexpectedly found that binge drinkers' state anxiety and heat pain threshold were positively correlated on the morning after drinking; therefore, more anxiety during alcohol withdrawal was associated with a hypoalgesic effect. Consequently, Experiments 1 and 2 showed that negative affect during alcohol withdrawal did not moderate alcohol withdrawal-induced hypersensitivity to pain. Experiment 1 showed greater alcohol craving in moderate and binge drinkers compared to abstainers. However, levels of craving were similar between moderate and binge drinkers. Specifically, this difference was seen in positive alcohol expectancy and having strong intent and making plans for drinking. Experiment 2 demonstrated that this elevated craving was not further enhanced by alcohol withdrawal and pain. Both moderate and binge drinkers consistently

showed higher levels of positive alcohol expectancy than the abstainers in Experiment 2, but different from Experiment 1, strong urges to drink with actual intent and plans were specific to binge drinkers.

Caveats of Evaluating Pain Sensitivity in Experiment 1 and Experiment 2

Before going over the findings from Experiment 1 and Experiment 2, understanding differences between the two experimental designs are important. Experiment 1 evaluated pain sensitivity during alcohol withdrawal after naturalistic drinking, but depended on their self-report and recall of recent drinking episode. Experiment 2 compared pain sensitivity before and after consumed alcohol up to a BAC of 0.08%, the NIAAA's definition of binge drinking. Although Experiment 2 intended to assess alcohol withdrawal-induced hyperalgesia in a controlled setting, a BAC of 0.08% seemed to be below the most binge drinker's typical drinking. Consequently, most binge drinkers in Experiment 2 reported less severe hangover symptoms after laboratory alcohol administration than their typical morning after drinking and their hangover symptoms were less severe than binge drinkers with acute alcohol consumption in Experiment 1. Additionally, the study hypothesis was blinded to all participants in Experiment 1, but it was only blinded to abstainers in Experiment 2. These caveats should be kept in mind during the following discussion.

Hypersensitivity to Muscle Pain during Alcohol Withdrawal

In Experiment 1, binge drinkers showed a lower muscle pressure pain threshold, which was reduced further during alcohol withdrawal. However, cutaneous mechanical

and heat pain thresholds were not changed. These results suggest that alcohol withdrawal might induce hypersensitivity to muscle pressure pain, but would not affect cutaneous mechanical and heat pain sensitivity in the early stages of binge drinking. Notably, this effect size was large (η^2 = .14). This is consistent with animal results showing reduced pressure pain threshold during alcohol withdrawal (6,17,18), but inconsistent in that animal results also show reduced mechanical and heat pain thresholds during alcohol withdrawal (8,18,60). Muscle pain can be observed in neuropathy (61) and muscle ache is a common hangover symptom (62). Known causes for alcoholic neuropathy are nutritional deficiency (e.g., thiamine), alcohol's neurotoxicity, or its metabolites (i.e., acetaldehyde) (63). However, the pathogenesis is less defined for alcohol withdrawal-induced hypersensitivity to muscle pain.

Animal studies have found that the SAM (e.g., epinephrine) and HPA (e.g., cortisol) stress axes play a critical role in alcohol withdrawal-induced muscle hyperalgesia (17). Animal research indicates that epinephrine and cortisol fully mediate the development and maintenance of alcohol withdrawal-induced hyperalgesia (17). The current study demonstrated that some of these animal findings can be translated into humans - epinephrine was elevated in binge drinkers, but alcohol withdrawal did not further increase epinephrine levels. Although additional pharmacological research is needed to determine whether epinephrine mediates withdrawal-induced hyperalgesia in humans (e.g., using β-adrenergic antagonist), the current results suggest that basal epinephrine levels alone explain a small portion (7%) of alcohol withdrawal induced

hyperalgesia. Different from the animal findings, the current study did not find any changes in cortisol.

According to the animal findings, alcohol withdrawal in the early stages of alcohol intoxication activates both the SAM and HPA axes and is involved in the induction and maintenance of alcohol withdrawal-induced hyperalgesia with a continued ethanol binge diet (17). Alcohol-withdrawal induced hyperalgesia was observed even at 1 week after cessation of the ethanol binge diet, but the role of epinephrine was not examined for this extended period of abstinence (17). In an animal study investigating the development and maintenance of stress-induced hyperalgesia, activation of both the SAM and HPA axes are necessary for the induction (64), but the SAM (epinephrine) axis is found to play a key role in its maintenance up to 2 weeks after exposure to stress (65). Therefore, persistent elevation of epinephrine levels after several years of binge drinking may be critical in the maintenance phase of alcohol withdrawal-induced hyperalgesia.

Over time, repeated episodes of alcohol withdrawal and activation of stress axes may cause hyperalgesia. Therefore, the long-term changes in the dynamics of binge drinking and the stress adaptation process may explain the progressive changes in pain sensitivity. Our brain and body's adaptive systems respond to stressful events such as alcohol withdrawal. Elevated epinephrine and cortisol responses during the initial episodes of alcohol intoxication and withdrawal are adaptive responses that work together with the other adaptive systems (e.g., the nervous, endocrine, immune systems) to regain the baseline stability (*homeostasis*) (66). Binge drinking in combination with other psychological and physical stressors, activate centrally organized adaptive systems to

maintain stability through changes (*allostasis*). After undergoing multiple stress challenges (*allostatic load* and repeated cycles of intoxication and withdrawal), these stress adaptive systems become dysregulated, and the dysregulated responses can be either "persistent" or "inadequate" (67). Human participants in the current study showed a "persistent" elevation of epinephrine after 2-4 years of drinking, while their epinephrine and cortisol responses were blunted during acute alcohol withdrawal. Therefore, the responses of the SAM and HPA stress systems may become "inadequate" during acute alcohol withdrawal. The current study did not find changes in basal cortisol levels in binge drinkers. Perhaps, binge drinkers' relatively normal basal cortisol levels and their unresponsiveness to the binge drinking episode represent a form of allostatic load, whereby the HPA system becomes "inadequate" while the SAM system exhibits a persistent up regulation in responses to the physical stress of repeated episodes of alcohol intoxication and withdrawal. Here stress hormones were discussed as a potential mechanism involved in alcohol withdrawal-induced neuropathic pain because alcohol is believed to cause neuropathic pain. However, muscle damage (necrosis) from alcohol intoxication may induce or partially contribute to this hyperalgesia because muscle necrosis occurs in acute and chronic alcohol-induced myopathy that manifests with muscle pain (68,69).

The binge drinkers in Experiment 1 and 2 were non-clinical healthy young adults with a relatively short history of drinking (i.e., about 2 years in Experiment 1 and 4 years in Experiment 2). Therefore, hypersensitivity to pressure pain may occur in advance of the development of clinical neuropathic pain. Clinically, this finding suggests that clinical

neuropathic pain may begin with further worsening pressure pain sensitivity or development of hypersensitivity to cutaneous mechanical and heat pain, reflecting sensory neuropathic pain. Additionally, the current experiment found that not all binge drinkers showed hypersensitivity to pressure pain. Similarly, previous studies report that alcoholic neuropathy develops in up to 49% of chronic alcoholics (3). Therefore, those who show hypersensitivity to muscle pain even in the early stages of binge drinking may be vulnerable to alcoholic neuropathic pain with prolonged binge drinking. A prospective study is needed to examine this possibility.

Capsaicin-induced Pain and Flare during Alcohol Withdrawal

In Experiment 2, capsaicin-induced spontaneous pain did not differ between the groups. However, exploratory pattern analyses found a J-shape curve relationship between the number of weekend drinks and peak pain intensity during capsaicin application. About a half drink was associated with slight reduction in peak pain intensity. Indeed, this pattern was also found in Experiment 1 with pressure pain thresholds. Moderate drinkers showed an increase in pressure pain thresholds whereas binge drinkers showed a decrease. This J-shape curve has been reported in cardiovascular health, stroke, type II diabetes, and all-cause mortality (70,71,72,73). Consequently, small amount of alcohol may have some analgesic effect against inflammatory-mediated pain and muscle pain.

Although capsaicin-induced pain intensity did not differ between the groups, size and intensity of capsaicin-induced flare were increased after binge drinking. Consequently, alcohol withdrawal may induce the release of the proinflammatory cytokines, (e.g., interleukin-6). Because elevated interleukin-6 levels can induce persistent

muscle hyperalgesia (74), Interleukin-6 mediated-persistent hyperalgesia in alcoholic neuropathy may be possible. Yet, the early stages of binge drinkers in the current study showed temporal enhancement of flare responses during alcohol withdrawal, but showed no persistent changes in the absence of binge drinking for at least three days.

Central Sensitization during Alcohol Withdrawal

Theoretically, alcohol withdrawal can alter central pain processing because CNS hyperexcitability is one of the alcohol withdrawal symptoms. However, CNS hyperexcitability is uncommon in the early stages of binge drinking. This might be a reason for the absence of a group difference in capsaicin-induce allodynia in the current study. Additionally, less hangover symptoms from laboratory alcohol administration might contribute to the null results. Consequently, a future study should examine whether central pain sensitization would emerge in long-term alcohol abusers without neuropathy.

The Role of Negative Affect in Alcohol Withdrawal-induced Hyperalgesia

Elevated levels of irritability, anxiety, and dysphoria are common symptoms of alcohol withdrawal. The current study measured the levels of state anxiety before and after a period of alcohol withdrawal as well as before and after pain testing. The binge drinkers in the current study showed no difference in state anxiety at the baseline, and in response to alcohol withdrawal and to laboratory pain testing. Additionally, anxiety-associated hyperalgesia to muscle pain was observed only in the abstainer group in Experiment 1. Unexpectedly, individual difference in alcohol withdrawal-induced anxiety was positively associated with cutaneous heat pain thresholds in the binge drinker group in Experiment

2. More increased anxiety during alcohol withdrawal was related to higher heat pain thresholds. In sum, negative affect did not exacerbate, but rather dampened withdrawal-induced hyperalgesia.

Issues in Translating Animal Findings to Humans

Some of our findings in humans are consistent with findings from previous animal models of binge drinking. Notably, withdrawal-induced muscle hyperalgesia occurred even in the early stages of binge drinking in humans, but cutaneous mechanical and heat pain as well as central pain processing remained unaffected by binge drinking. Additionally, binge drinkers' basal epinephrine levels were elevated, but levels did not increase any further during alcohol withdrawal. Cortisol levels were not altered by binge drinking. Animal protocols of ethanol binge diets administer alcohol to rats more regularly and frequently (4 days on and 3 days off) for up to 4 weeks (6,17) and the protocols assessing withdrawal-induced hyperalgesia evaluate pain sensitivity after stopping 10 days of the continuous ethanol diet (8,60). Hence, animal studies demonstrate alcohol withdrawal-induced hyperalgesia in the early stages of a rigorous binge or excessive ethanol diet and this may reflect the mechanisms of induction. Additionally, animals were tested under homogenous conditions. For example, laboratory animals have the same genetic makeup, controlled diet, no other health risk factors, and a controlled environment. In contrast, human participants have large variability in drinking patterns and other related physiological, psychological, genetic, and environmental factors. Human subjects in the current study reported that the frequency of binge drinking was about 2 to 4 times a month and the period of drinking was 2 to 4 years. Thus, our human subjects had more binge-

withdrawal cycles than used in the animal models, and the amount of alcohol consumed during each binge is likely to vary considerably between individuals. Clearly, the young adult binge drinkers in the current study were tested after a much longer period of alcohol use and patterns of drinking are likely to vary considerably between individuals. Additionally, several contributing factors co-exist in human binge drinkers and covary with alcohol use. For example, personality (i.e., sensation-seeking tendency), psychological conditions (i.e., depression), health risk behaviors (smoking, other substance use), life stressors, and coping (substance use as a coping strategy) may also affect alcohol abuse and pain sensitivity. Consequently, these multilevel and multi-system factors should be considered when attempting to understanding the mechanisms of alcohol withdrawal-induced hyperalgesia in humans.

Pain and Craving

Alcohol produces acute analgesic and delayed onset hyperalgesic effects. This time-dependent opposing process suggests a possibility of pain as being a motivation for drinking despite the fact that alcohol progressively worsens pain with persistent use (21). The current experiment indirectly examined this possibility by measuring changes in alcohol craving during withdrawal and after laboratory pain testing. The current study found that elevated alcohol craving was characteristics of both moderate and binge drinkers, but was unaffected by alcohol withdrawal and pain in the young adult binge drinkers. This null finding may be due to the nature of the laboratory pain, which is relatively brief, temporary, and less intense than clinical pain, which is often persistent, uncontrollable, and severe. Alternatively, the alcohol dose administered in the laboratory

may be insufficient to induce withdrawal symptoms and consequent craving. Importantly, the current results do not rule out the possibility that alcohol withdrawal and pain can motivate drinking to directly reduce negative affect and pain during alcohol withdrawal. However, the current findings suggest that these negative reinforcing properties of alcohol may not accompany changes in craving.

Clinical Implications

The current study demonstrated that hypersensitivity to muscle pain and more intense inflammatory-mediated pain during alcohol withdrawal occurred even in young adult binge drinkers who were otherwise healthy. This information can be delivered to all drinkers as a health warning. Additionally, elevated epinephrine levels in binge drinkers suggest that stress management may benefit binge and heavy drinkers by lowering the risk of stress related health problems. Lastly, QST, especially for pressure pain threshold, may be useful to identify individual with subclinical and early stages of alcoholic neuropathy or to prescreen vulnerable individuals for neuropathy.

Limitations and Future Studies

Experiment 1 compared pain sensitivity between the groups after naturally occurring drinking episodes. Consequently, this experiment was unable to recruit the target number of participants with acute alcohol use. Additionally, the abstainer and moderate drinker groups included an equal number of men and women whereas the group of binge drinkers without acute alcohol included more women and binge drinkers with acute alcohol use included more men. Future research should examine potential gender

differences in alcohol withdrawal-induced hyperalgesia. Lastly, this between subject-design was limited in examining the direct effect of alcohol withdrawal on pain sensitivity because of unknown individual pain sensitivity at baseline.

The critical limitation of Experiment 2 was the alcohol administration protocol because it failed to induce withdrawal symptoms the morning after binge drinking that were comparable to the individuals' typical withdrawal symptoms. This suggests that laboratory alcohol administration targeting a BAC of 0.08% may be insufficient for investigating this withdrawal effect in binge drinkers. Additionally, some moderate and binge drinkers anticipated analgesic effects of alcohol. This preconception by some subjects of an analgesic effect may have influenced the QST results.

Conclusion

The current experiment demonstrated that alcohol withdrawal-hypersensitivity to muscle pain occurred in young adult binge drinkers with a relatively short history of drinking. Additionally, elevated basal levels of epinephrine were observed in binge drinkers, but levels did not change during alcohol withdrawal. Basal epinephrine contributed to withdrawal-induced hyperalgesia to a small degree, but cortisol did not play a role. The role of anxiety was minimal or associated with hypoalgesia during alcohol withdrawal. Finally, laboratory pain and alcohol withdrawal did not increase alcohol craving. Instead, elevated alcohol craving was a characteristic of young adult drinkers compared to abstainers.

9. REFERENCES

1. Center for Behavioral Health Statistics and Quality. Behavioral health trends in the United States and health (HHS Publicatin No. SMA 15-4927, NSDUH Series H-50), 2015. Retrived from http://www.samhsa.gov/data/ on 5/1/2016.
2. U.S. Dept. of Health and Human Services. 10th special report to the US Congress on alcohol and health: highlights from current research, 2000. Retrived from http:// pubs.niaaa.nih.gov/publications/10Report/10thSpecialReport.pdf on 5/1/2016.
3. Vittadini G, Buonocore M, Colli G, Terzi M, Fonte R, Biscaldi G. Alcoholic polyneuropathy: a clinical and epidemiological study. Alcohol Alcoholism 2001;36(5):393-400.
4. Wolff H, Hardy J, Goodell H. Studies on pain: measurement of the effect of ethyl alcohol on the pain threshold and on the "alarm" reaction. J Pharmacol Exp Ther 1942;75(1):38-49.
5. Gatch MB. Ethanol withdrawal and hyperalgesia. Curr Drug Abuse Rev 2009;2(1): 41-50.
6. Dina OA, Messing RO, Levine JD. Ethanol withdrawal induces hyperalgesia mediated by PKCε. Eur J Neurosci 2006;24(1):197-204.
7. Jochum T, Boettger MK, Burkhardt C, Juckel G, Bär K-J. Increased pain sensitivity in alcohol withdrawal syndrome. Eur J Pain 2010;14(7):713-8.
8. Gatch MB, Lal H. Effects of ethanol and ethanol withdrawal on nociception in rats. Alcohol Clin Exp Res 1999;23(2):328-33.

9. Gameiro GH, Arthuri MT, Tambeli CH, Veiga MCFA. Effects of ethanol on deep pain evoked by formalin injected in TMJ of rat. Life Sci 2003;73(26):3351-61.
10. American Psychiatric Association. Diagnostic and statistical manual of mental disorders (5th ed.). Arlington, VA: American Psychiatric Publishing.
11. Bayard M, Mcintyre J, Hill KR, Woodside Jr J. Alcohol withdrawal syndrome. Am Fam Physician 2004;69(6):1443-50.
12. Hesse M, Tutenges S. Predictors of hangover during a week of heavy drinking on holiday. Addiction 2010;105(3):476-83.
13. Howland J, Rohsenow DJ, Allensworth-Davies D, Greece J, Almeida A, Minsky SJ, Arnedt JT, Hermos J. The incidence and severity of hangover the morning after moderate alcohol intoxication. Addiction 2008;103(5):758-65.
14. Collins WE, Chiles WD. Laboratory performance during acute alcohol intoxication and hangover. Hum Factors 1980;22(4):445-62.
15. Wiese JG, Shlipak MG, Browner WS. The alcohol hangover. Ann Intern Med 2000; 132(11):897-902.
16. Penning R, van Nuland M, AL Fliervoet L, Olivier B, C Verster J. The pathology of alcohol hangover. Curr Drug Abuse Rev 2010;3(2):68-75.
17. Dina OA, Khasar SG, Alessandri-Haber N, Green PG, Messing RO, Levine JD. Alcohol-induced stress in painful alcoholic neuropathy. Eur J Neurosci 2008;27(1):83-92.

18. Dina OA, Barletta J, Chen X, Mutero A, Martin A, Messing RO, Levine JD. Key role for the epsilon isoform of protein kinase C in painful alcoholic neuropathy in the rat. J Neurosci 2000;20(22):8614-9.
19. Joseph EK, Levine JD. Multiple PKCε-dependent mechanisms mediating mechanical hyperalgesia. Pain 2010;150(1):17-21.
20. Becker HC. Kindling in alcohol withdrawal. Alcohol Res Health 1998;22(1):25-33.
21. Egli M, Koob GF, Edwards S. Alcohol dependence as a chronic pain disorder. Neurosci Biobehav Rev 2012;36(10):2179-92.
22. Apkarian AV, Neugebauer V, Koob G, Edwards S, Levine JD, Ferrari L, Egli M, Regunathan S. Neural mechanisms of pain and alcohol dependence. Pharmacol Biochem Behav 2013;112:34-41.
23. Koob GF, Le Moal M. Addiction and the brain antireward system. Annu Rev Psychol 2008;59:29-53.
24. Gharat L, Szallasi A. Medicinal chemistry of the vanilloid (Capsaicin) TRPV1 receptor: Current knowledge and future perspectives. Drug Dev Res 2007;68(8):477-97.
25. LaMotte RH, Shain CN, Simone DA, Tsai E. Neurogenic hyperalgesia: psychophysical studies of underlying mechanisms. J Neurophysiol 1991;66(1):190-211.
26. Torebjörk H, Lundberg L, LaMotte R. Central changes in processing of mechanoreceptive input in capsaicin-induced secondary hyperalgesia in humans. J Physiol 1992;448(1):765-80.

27. Collins RL, Parks GA, Marlatt GA. Social determinants of alcohol consumption: the effects of social interaction and model status on the self-administration of alcohol. J Consult Clin Psychol 1985;53(2):189-200.
28. Cohen S. Perceived stress in a probability sample of the United States. In Spacapan S, Oskamp S.(eds). The Social Psychology of Health. Newbury Park, CA, Sage, 1988, pp 31-67.
29. Antony MM, Bieling PJ, Cox BJ, Enns MW, Swinson RP. Psychometric properties of the 42-item and 21-item versions of the Depression Anxiety Stress Scales in clinical groups and a community sample. Psychol Assessment 1998;10(2):176-181.
30. Lovibond SH, Lovibond PF. Manual for the Depression Anxiety Stress Scales. 2 ed. Sydney: Psychology Foundation; 1995.
31. Singleton E, Tiffany S, Henningfield J, editors. Development and validation of a new questionnaire to assess craving for alcohol: problems of drug dependence. Proceeding of the 56th Annual Meeting, The College on Problems of Drug Dependence National Institute on Drug Abuse, Rockville, MD; 1994.
32. Patton JH, Stanford MS, Barratt ES. Factor structure of the barratt impulsiveness scale. J Clin Psychol 1995;51(6):768-74.
33. Slutske WS, Piasecki TM, Hunt-Carter EE. Development and initial validation of the Hangover Symptoms Scale: prevalence and correlates of hangover symptoms in college students. Alcohol Clin Exp Res 2003;27(9):1442-50.

34. Rohsenow DJ, Howland J, Minsky SJ, Greece J, Almeida A, Roehrs TA. The Acute Hangover Scale: A new measure of immediate hangover symptoms. Addict Behav 2007;32(6):1314-20.
35. Marteau TM, Bekker H. The development of a six-item short-form of the state scale of the Spielberger State-Trait Anxiety Inventory (STAI). Brit J Clin Psychol 1992; 31(3):301-6.
36. Bradley MM, Lang PJ. Measuring emotion: the self-assessment manikin and the semantic differential. J Behav Ther Exp Psy 1994;25(1):49-59.
37. Watson D, Clark LA, Tellegen A. Development and validation of brief measures of positive and negative affect: the PANAS scales. J Pers Soc Psychol 1988;54(6):1063-70.
38. Chopra K, Tiwari V. Alcoholic neuropathy: possible mechanisms and future treatment possibilities. Brit J Clin Pharmaco 2012;73(3):348-62.
39. Koike H, Iijima M, Sugiura M, Mori K, Hattori N, Ito H, et al. Alcoholic neuropathy is clinicopathologically distinct from thiamine-deficiency neuropathy. Ann Neurol 2003;54(1):19-29.
40. Rolke R, Baron R, Maier C, Tölle TR, Treede RD, Beyer A, et al. Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): Standardized protocol and reference values. Pain 2006;123(3):231-43.
41. Rolke R, Magerl W, Campbell KA, Schalber C, Caspari S, Birklein F, et al. Quantitative sensory testing: a comprehensive protocol for clinical trials. Eur J Pain 2006;10(1):77-88.

42. Hagander LG, Midani HA, Kuskowski MA, Parry GJG. Quantitative sensory testing: effect of site and skin temperature on thermal thresholds. Clin Neurophysiol 2000; 111(1):17-22.
43. Hopper PD, Bradford JL. Understanding the endocrine system. In. Williams LS, Hopper PD. (Eds). Understanding Medical Surgical Nursing, 5th edition: FA Davis; 2015, pp 871-1448.
44. Ruha A, Sallinen S, Nissilä S. A real-time microprocessor QRS detector system with a 1-ms timing accuracy for the measurement of ambulatory HRV. IEEE Trans Biomed Eng 1997;44(3):159-67.
45. Tabachnick BG, Fidell LS, Osterlind SJ. Using multivariate statistics, 5th edition. Boston, MA, Pearson Education, Inc. 2001.
46. Fritz MS, MacKinnon DP. Required sample size to detect the mediated effect. Psychol Sci 2007;18(3):233-9.
47. Whiteside SP, Lynam DR, Miller JD, Reynolds SK. Validation of the UPPS impulsive behaviour scale: a four-factor model of impulsivity. Eur J Pers 2005;19(7):559-74.
48. Zuckerman M. Zuckerman-Kuhlman Personality Questionnaire (ZKPQ): an alternative five-factorial model. In De Raad B, Perugini M. (eds). Big Five Assessment 2002:377-96.
49. Muro A, Albiol S. Zuckerman-kuhlman personality questionnaire: psychometric properties in a sample of general population. Psychol Rep 2008;103(3):845-56.

50. Martin CS, Earleywine M, Musty RE, Perrine MW, Swift RM. Development and validation of the Biphasic Alcohol Effects Scale. Alcohol Clin Exp Res 1993;17(1): 140-6.
51. Gomez A, Conde A, Santana J, Jorrin A. Diagnostic usefulness of brief versions of Alcohol Use Disorders Identification Test (AUDIT) for detecting hazardous drinkers in primary care settings. J Stud Alcohol 2005;66(2):305-8.
52. Babor TF, Higgins-Biddle JC, Saunders JB, Monteiro MG. AUDIT: The Alcohol Use Disorders Identification Test: guidelines for use in primary care: World Health Organization. Department of Mental health and Substance Dependence. 2001. Retrived from http://www.talkingalcohol.com/files/pdfs/WHO_audit.pdf on 5/10/2016.
53. Fleming M, Barry K, MacDonald R. The alcohol use disorders identification test (AUDIT) in a college sample. Int J Addict 1991;26(11):1173-85.
54. Price DD, McGrath PA, Rafii A, Buckingham B. The validation of visual analogue scales as ratio scale measures for chronic and experimental pain. Pain 1983;17(1):45-56.
55. Green AQ, Krishnan ST, Rayman G. C-fiber function assessed by the laser doppler imager flare technique and acetylcholine iontophoresis. Muscle Nerve 2009;40(6): 985-91.
56. Green AQ, Krishnan S, Finucane FM, Rayman G. Altered C-fiber function as an indicator of early peripheral neuropathy in individuals with impaired glucose tolerance. Diabetes Care 2010;33(1):174-6.

57. Wetherill RR, Fromme K. Acute alcohol effects on narrative recall and contextual memory: An examination of fragmentary blackouts. Addicti Behav 2011;36(8):886-9.
58. Matthews DB, Miller WR. Estimating blood alcohol concentration: two computer programs and their applications in therapy and research. Addict Behav 1979;4(1):55-60.
59. Cathcart S, Pritchard D. Reliability of pain threshold measurement in young adults. J Headache Pain 2006;7(1):21-6.
60. Gatch MB, Selvig M. Theophylline blocks ethanol withdrawal-induced hyperalgesia. Alcohol Alcoholism. 2002;37(4):313-7.
61. Marchettini P, Formaglio F, Lacerenza M. Clinical interpretations of intraneural muscle nociceptors recordings in humans. J Musculoskelet Pain 1999;7(1-2):55-9.
62. Swift R, Davidson D. Alcohol hangover. Alcohol Health Res World. 1998;22:54-60.
63. Koike H, Sobue G. Alcoholic neuropathy. Curr Opin Neurol 2006;19(5):481-6.
64. Khasar SG, Burkham J, Dina OA, Brown AS, Bogen O, Alessandri-Haber N, Green PG, Reichling DB, Leving JD. Stress induces a switch of intracellular signaling in sensory neurons in a model of generalized pain. J Neurosci 2008;28(22):5721-30.
65. Khasar SG, Dina OA, Green PG, Levine JD. Sound stress-induced long-term enhancement of mechanical hyperalgesia in rats is maintained by sympathoadrenal catecholamines. J Pain 2009;10(10):1073-7.
66. Koob GF, Le Moal M. Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology 2001;24(2):97-129.

67. Juster R-P, McEwen BS, Lupien SJ. Allostatic load biomarkers of chronic stress and impact on health and cognition. Neurosci Biobehav Rev 2010;35(1):2-16.

68. Song SK, Rubin E. Ethanol produces muscle damage in human volunteers. Science 1972;175(4019):327-8.

69. Urbano-Márquez, A. and Fernández-Solà, J. Effects of alcohol on skeletal and cardiac muscle. Muscle Nerve 2004;30(6), 689-707.

70. O'Keefe JH, Bybee KA, Lavie CJ. Alcohol and cardiovascular health: the razor-sharp double-edged sword. J Am Coll Cardiol 2007;50(11):1009-14.

71. Koppes LL, Dekker JM, Hendriks HF, Bouter LM, Heine RJ. Moderate alcohol consumption lowers the risk of type 2 diabetes: a meta-analysis of prospective observational studies. Diabetes Care 2005;28(3):719-25.

72. Di Castelnuovo A, Costanzo S, Bagnardi V, Donati MB, Iacoviello L, de Gaetano G. Alcohol dosing and total mortality in men and women: an updated meta-analysis of 34 prospective studies. Arch Intern Med 2006;166(22):2437-45.

73. Rehm J, Greenfield TK, Rogers JD. Average volume of alcohol consumption, patterns of drinking, and all-cause mortality: results from the US National Alcohol Survey. Am J Epidemiol 2001;153(1):64-71.

74. Dina OA, Green PG, Levine JD. Role of interleukin-6 in chronic muscle hyperalgesic priming. Neuroscience 2008;152(2):521-5.